Instruction Manual

MICROX TX3

fiber-optic oxygen meter

Loligo Systems ApS • Niels Pedersens Allé 2 • DK-8830 Tjele • Denmark • Phone +45 89 99 25 45
Fax +45 89 99 25 99 • www.loligosystems.com • mail@loligosystems.com • VAT-no. 2746 7849 • Bank 5984 0004001376

Instruction Manual
Microx TX3

Software Version TX3v531

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Specification of Microx TX3:

PC-controlled one-channel fiber-optic oxygen meter for oxygen microsensors;

excitation wavelength of 505 nm;

quartz-quartz glass-fibers of 140 µm outer diameter connected by ST-fiber connectors;

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1 Preface

Congratulations!

You have chosen a new innovative technology for measuring oxygen!

The **Microx TX3** is a precise single channel, **temperature compensated oxygen meter** specially developed for very small fiber-optic oxygen microsensors (tip diameter < 50 µm).

The small outer dimensions, low power consumption and a robust box make it ready for indoor and **outdoor** applications.

The Microx TX3 was specially developed for very small fiber-optic oxygen microsensors. It is based on a **novel technology**, which creates very stable, internal referenced measured values. This enables a more flexible use of oxygen sensors in many different fields of interest.

Optical oxygen microsensors (also called optodes) have the following outstanding properties:

- They do not consume oxygen
- Their signal does not depend on the flow rate of the sample
- Diameter of the micro-sensor tip is < 50 µm
- Fast response time better than 1 s
- Measures oxygen in both liquids and the gas phase
- On-line temperature-compensation of the oxygen content

Therefore, they are ideally suited for the examination of very small sample volumes and for measuring oxygen gradients with high spatial resolution in heterogeneous systems. Their small dimensions even allow measurements in living systems.

A set of different microsensors is available to make sure to meet your requirements for you applications.

Please feel free to contact our service team to find the best solution for your application.

Your Loligo Team

2 Safety Guidelines

PLEASE READ THESE INSTRUCTIONS CAREFULLY BEFORE WORKING WITH THIS INSTRUMENT!

This device has left our works after careful testing of all functions and safety requirements.

The perfect functioning and operational safety of the instrument can only be ensured if the user observes the usual safety precautions as well as the specific safety guidelines stated in these operating guidelines.

- Before connecting the device to the electrical supply network, please ensure that the operating voltage stated on the power supply corresponds to the mains voltage
- The perfect functioning and operational safety of the instrument can only be maintained under the climatic conditions specified in Chapter 10 "Technical Data" in this operating manual.
- If the instrument is moved from cold to warm surroundings, condense may form and interfere with the functioning of the instrument. In this event, wait until the temperature of the instrument reaches room temperature before putting the instrument back into operation.
- Balancing, maintenance and repair work must only be carried out by a suitable qualified technician, trained by us.
- Especially in the case of any damage to current-carrying parts, such as the power supply cable or the power supply itself, the device must be taken out of operation and protected against being put back into operation.
- If there is any reason to assume that the instrument can no longer be employed without a risk, it must be set aside and appropriately marked to prevent further use.
- The safety of the user may be endangered, e. g., if the instrument
    - is visibly damaged;
    - no longer operates as specified;
    - has been stored under adverse conditions for a lengthy period of time;
    - has been damaged in transport
- If you are in doubt, the instrument should be sent back to the manufacturer for repair and maintenance.
- The operator of this measuring instrument must ensure that the following laws and guidelines are observed when using dangerous substances:
    - EEC directives for protective labor legislation;
    - National protective labor legislation;
    - Safety regulations for accident prevention;
    - Safety data-sheets of the chemical manufacturer

The Microx TX3 is not protected against water spray;
The Microx TX3 is not water proof;
The Microx TX3 must not be used under environmental conditions which cause water-condensation in the housing;
The Microx TX3 is sealed;
The Microx TX3 must not be opened;

We explicitly draw your attention to the fact that any damage of the *manufactural* seal will render of all guarantee warranties invalid.

Any internal operations on the unit must be carried out by personal explicitly authorized by Loligo and under antistatic conditions.

Needle-type sensors are housed in extremely sharp syringe needles. Avoid injury by handling the needle carefully. Please pay attention to all safety guidelines for safe handling of sharp needles and syringes. Beware of injuring with the needle as well as with the sensor tip. The glass fiber can break if pricked into the skin and can cause inflammation.

The Microx TX3 may only be operated by qualified personal.
This measuring instrument was developed for use in the laboratory. Thus, we must assume that, as a result of their professional training and experience, the operators will know the necessary safety precautions to take when handling chemicals.

Keep the Microx TX3 and the equipment such as PT100, PT1000 temperature sensor, power supply and needle type sensors out of the reach of children!

As the manufacturer of the Microx TX3, we only consider ourselves responsible for safety and performance of the device if

- the device is strictly used according to the instruction manual and the safety guidelines
- the electrical installation of the respective room corresponds to the DIN IEC/VDE standards.

The Microx TX3 and the microsensors must not be used in vivo examinations on humans!

The Microx TX3 and the microsensors must not be used for human-diagnostic or therapeutically purposes!

3 Description of the Microx TX3 Device

The **Microx TX3** is a precision, ***temperature compensated***, oxygen meter, designed for fiber-optic oxygen microsensors.

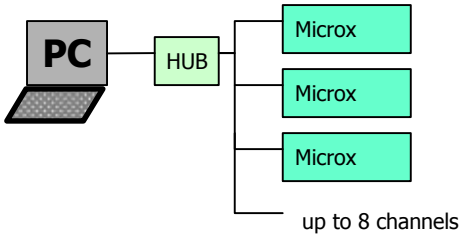
The small outer dimensions, low power consumption and robust box makes it ready for indoor and **outdoor** application.

For data visualization and storage the instruments have to be connected to a PC computer.
.

Microx TX3 instruments features:

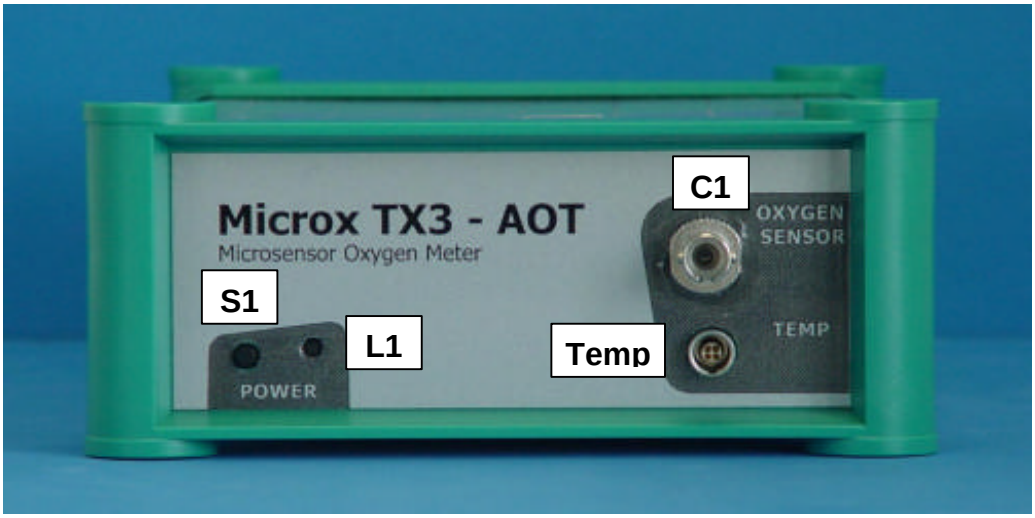
- high precision
- portable (battery power optional)
- analog/digital data output (on request)
- temperature compensation

There also exits the possibility to combine several single Microx TX3 oxygen meter to obtain a multichannel system. It allows the user to create a 2, 3, 4 or more channel system.



The Microx TX3 oxygen meter contains a dual 12 bit analog output and an external trigger input. The analog output values can be programmed with the PC software (included). The user can choose between oxygen, temperature, amplitude or phase for each channel independently. Microx TX3 can be used as a stand-alone instrument when combined with an external data logger.

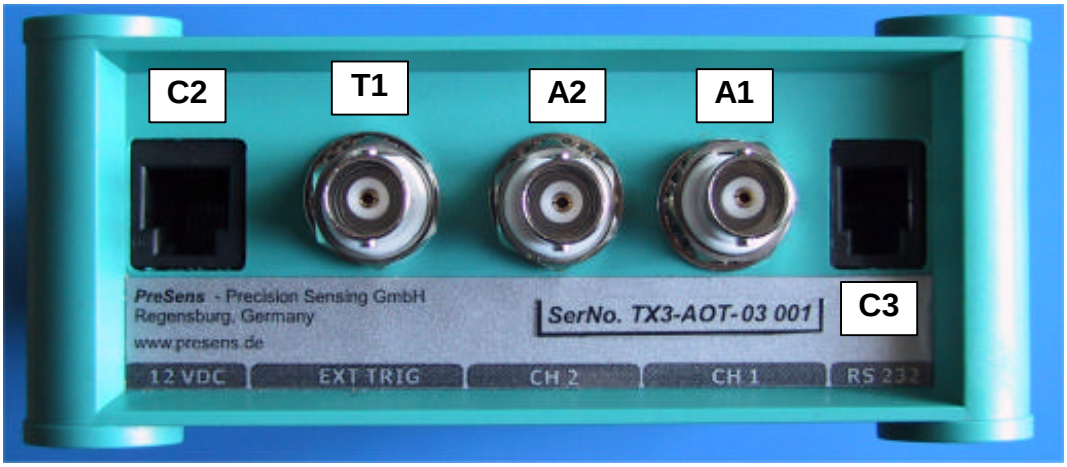
Front Panel



ELEMENT	DESCRIPTION	FUNCTION
S1	ON/OFF switch	Switches the device ON and OFF
C1	ST fiber connector	Connect the fiber-optic oxygen microsensor here.
L1	Control LED	red: instrument off; green: instrument on; orange: stand by;
Temp	Connector for PT 1000 temperature sensor	Connect the PT 1000 temperature sensor for temperature compensated measurements here.

Rear Panel of the Microx TX3 device

Two standard BNC connectors are added for analog output channels 1 and 2, another one for external trigger input. See figure below.
The electrical specifications of all rear panel connectors are given in technical specification sheet. Please read also the technical notes to avoid mistakes.



ELEMENT	DESCRPTION	FUNCTION
C2	Line adapter for power supply	Connector for 9 - 36 V DC power supply.
C3	RS232 interface (male)	Connect the device with a RS232 data cable to your PC/Notebook here.
A1	Analog out (channel 1)	Connect the device with external devices, e.g. a data logger
A2	Analog out (channel 2)	Connect the device with external devices, e.g. a data logger
T1	External trigger input	Connect the device with external devices, e.g. data logger with a trigger output, pulse generator

Features

- microsensor oxygen meter with temperature compensation
- 2 x 12bit, programmable analog channels, with galvanic isolation
- measuring range 0 - 500% air saturation
- 9 – 36 V supply voltage (or 220/110V AC adaptor)
- RS 232 interface
- robust metal box

4 Required Basic Equipment

- Oxygen meter Microx TX3*
- Software for Microx TX3*
- PC / Notebook
  (System requirements:
  Windows 95/98/2000/Millenium/NT 4.0/XP; Pentium processor, at least 133 MHz, 16 MB RAM)
- RS 232 Cable *
- Line adapter (110 - 220 V AC, 12 V DC) *
- Temperature sensor PT 1000*
- Oxygen-sensitive microsensor
  The microsensors are mounted into different types of housings
- Vessels for calibration standard 100 (water-vapor saturated air ≡ 100 % air saturation) and calibration solution 0 (oxygen-free water)
  We recommend Schott laboratory bottles with a thread which can be obtained by VWR International (ordering number: 215L1515)
- Laboratory support with clamp, micro-manipulator

*: scope of supply

5 Fiber-Optic Oxygen Microsensors: Sensors and Housings

5.1 Oxygen-Sensitive Microsensors

5.1.1 Sensor Characteristic

The principle of the sensor operation is based on the quenching of luminescence caused by collision between molecular oxygen and luminescent dye molecules in the excited state. Figure 5.1 shows a typical response curve of an oxygen-sensitive microsensor. In the presence of oxygen the signal - in our case the phase angle Φ - decreases. The phase angle Φ can be related to the oxygen content as shown in Figure 5.2. The theoretical aspects are explained more detailed in the appendix.

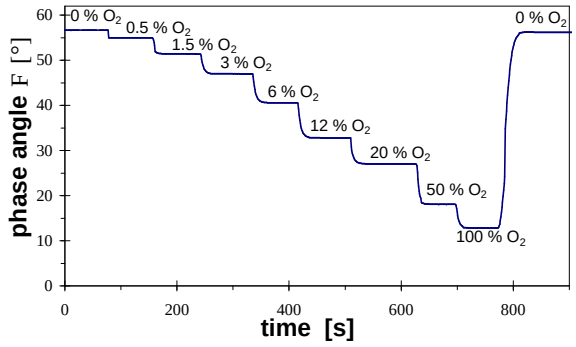


Figure 5.1 Response of an oxygen microsensor toward changes in the oxygen concentration.

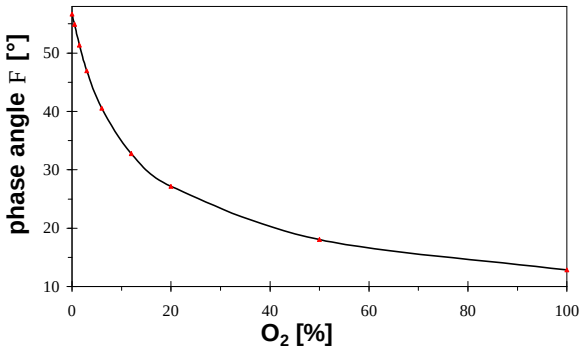


Figure 5.2 Effect of the phase angle of an oxygen microsensor on different oxygen contents

Specifications of the oxygen microsensor and the Microx TX3

The optimal measuring range of the oxygen-sensitive microsensors is from 0 to 50 % pure oxygen (=250 % air-saturation). However, it is also possible to measure oxygen up to 100%. Please contact the Loligo service team to get the appropriate software when measuring up to 100 % oxygen.
Table 5.1 displays the measuring range of microsensors in different oxygen units.

Table 5.1 Specifications of the OXY-4 micro device and the respective oxygen microsensors..

INSTRUMENT			
Channels	1 x optical channel (ST connector, Core/Center 100/140) , designed for micro-sensors; 1 x PT100/PT1000 connector		
	Dissolved Oxygen	**Gaseous Oxygen**	
Measurement range	0 - 45 mg/L (ppm) 0 - 1.4 mmol	0 - 500 % air-sat. 0 - 100 % oxygen-sat. 0 - 760 Torr 0 - 1013 hPa	
Limit of Detection (LOD)	0.2 % air-saturation, 20 ppb dissolved oxygen	0.41 hPa, 0.30 Torr	
Resolution at 20 °C and 1013 hPa	0.09 ± 0.005 mg/L (ppm) 2.72 ± 0.01 mg/L (ppm) 9.06 ± 0.04 mg/L (ppm) 22.65 ± 0.17 mg/L (ppm)	1 ± 0.05 % air-sat. 30 ± 0.13 % air-sat. 100 ± 0.44 % air-sat. 250 ± 1.8 % air-sat.	
		0.21 ± 0.01 % oxygen 6.3 ± 0.03 % oxygen 20.9 ± 0.09 % oxygen 52.4 ± 0.38 % oxygen	
	2.83 ± 0.14 µmol 85.0 ± 0.3 µmol 283.1 ± 1.3 µmol 798.0 ± 5.2 µmol	1.55 ± 0.08 Torr 46.7 ± 0.20 Torr 155.5 ± 0.65 Torr 388.8 ± 2.65 Torr	
		2 ± 0.1 hPa *60* ± 0.26 hPa 200 ± 0.87 hPa 500 ± 3.54 hPa	
Response time (t_{90}) for tapered sensors	< 1 s, without optical isolation < 5 s, with optical isolation	< 0.5 s, without optical isolation < 1 s, with optical isolation	
Response time (t_{90}) for flat-broken sensors	< 15 s, without optical isolation < 30 s, with optical isolation	< 5 s, without optical isolation < 10 s, with optical isolation	
Accuracy at 100 % air-sat. (20 °C)	± 1% at 100 % air-saturation; ± 0.15% at 1 % air-saturation		
Temperature (PT1000)	**Range**	**Resolution**	**Accuracy**
	0-50°C	±0.5°C	±1°C
Digital interface	RS 232 interface with galvanic isolation (19200 Baud, Databits 8), RJ connector (RJ 6/4 - SUB-D 9 cable for RS232 PC port included)		
Analog outputs	Dual 12bit programmable outputs with galvanic isolation (BNC connectors)		
Specification	**Range**	**Resolution**	**Accuracy**
Analog output (Oxygen)	0-400% air-sat.	±0.2% air-sat.	±0.5% air-sat.
Analog output (Temperature, PT1000)	0-50°C	±0.2°C	±1°C
External Trigger	TTL compatible with galvanic isolation (BNC connector)		
Acquisition time	Programmable (default 1 second, min: 250 ms)		
Power supply	12 VDC/max.550 mA (110-240 VAC, 50/60 Hz adapter)		
SOFTWARE OxyView			
Oxygen units	User selectable from: air-saturation, oxygen-saturation, hPa, Torr, mg/L (=ppm), µmol		
Compatibility	Windows 95/98/2000/Millenium/NT4.0/2000/XP		
Calibration	Conventional two-point calibration with oxygen-free environment (nitrogen, sodium sulfite) and air-saturated environment		
ENVIRONMENTAL CONDITIONS			
Operating Temperature (°C)	0 to +50		
Storage Temperature (°C)	-10 to +65		
Relative humidity (%)	up to 95		
Dimensions, DxWxH (mm)	185 x 110 x 45		
Weight (kg)	0.63		

Temperature:

Microsensors can be used in the temperature range of -10 to 80 °C. We offer a Pt1000 temperature sensor in combination with the Microx TX3 to record temperature variations which are compensated using the Microx TX3 software (see Chapter 7, ***Calibration of Microsensors*** and Chapter 8, ***Measurement***). In the appendix you will find a detailed description of the ***Temperature Dependent Constants Affecting the Oxygen Content*** (chapter 13.4) and ***Temperature Compensation of the Response of Oxygen Sensors*** (chapter 13.5).

Cross sensitivity:

There exists no cross sensitivity for carbon dioxide (CO_2), hydrogen sulfide (H_2S), ammonia (NH_3), pH, any ionic species like sulfide (S_2^-), sulfate (SO_4^{2-}), chloride (Cl^-) or salinity. Turbidity and changes in the stirring rate have no influence on the measurement.

The sensors can also be used in methanol- and ethanol-water mixtures as well as in pure methanol and ethanol.

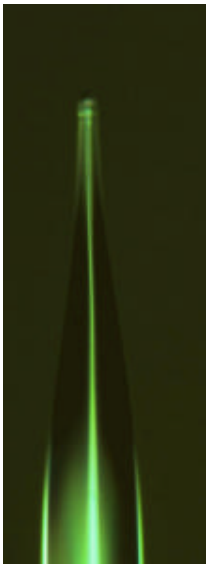
We recommend to avoid other organic solvents, such as acetone, chloroform or methylene chloride, which may swell the sensor matrix.

Interferences were found for gaseous sulfur dioxide (SO_2) and gaseous chlorine (Cl_2). Both of them mimic higher oxygen concentrations.

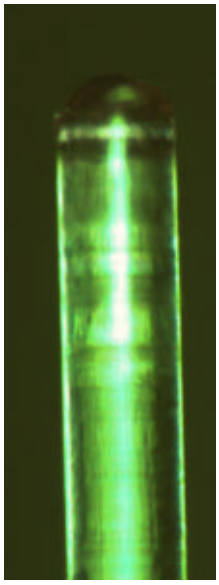
5.1.2 Design of the Sensor Tips

All the sensors mounted in different housings are available with two different glass-fiber tips, (**A**) a < 50 µm tapered tip and (**B**) a 140 µm flat-broken tip.

(**A**) ***Tapered sensor tip***



(**B**) ***Flat-broken sensor tip***



Advantages of microsensors with a tapered tip

- high spatial resolution (<50 µm)
- very fast response times (t_{90} up to 1 s in the liquids and < 0.2 s in the gas phase)

Disadvantages of microsensors with a tapered tip:

- fragile
- display photobleaching

Advantages microsensors with a flat-broken tip

- more photostable than tapered ones ➔ long-term stable
- more robust

Disadvantages of microsensors with flat-broken tip

- response times (t_{90}) in the order of 30 s

Response time

The response time (t_{90}, 90 % of the signal change has occurred) in water is dependent from the sensor tip size and typical ranges from 1s (for a sensor tip tapered < 50 µm) to about 20 s for a flat broken 140 µm tip (see figure below).

Unlike electrodes, optical sensors do **not** consume oxygen and the signal is independent of changes in flow velocity which means that stirring decreases the response time, but has no effect on the measured value.

Optical isolation of the oxygen-sensitive layer which is applied to exclude ambient light and improve chemical resistance will slow down the sensor response.

	Dissolved oxygen	Gaseous oxygen
Response time (t_{90}) for		
tapered sensors (tip diameter < 50 µm)	1 s; (< 5 s with O.I.*)	< 0.5 s; (< 1 s with O.I.*)
flat-broken sensors (tip diameter 150 µm)	< 15 s; (< 30 s with O.I.*)	< 5 s; (< 10 s with O.I.*)

*O.I.: Optical Isolation

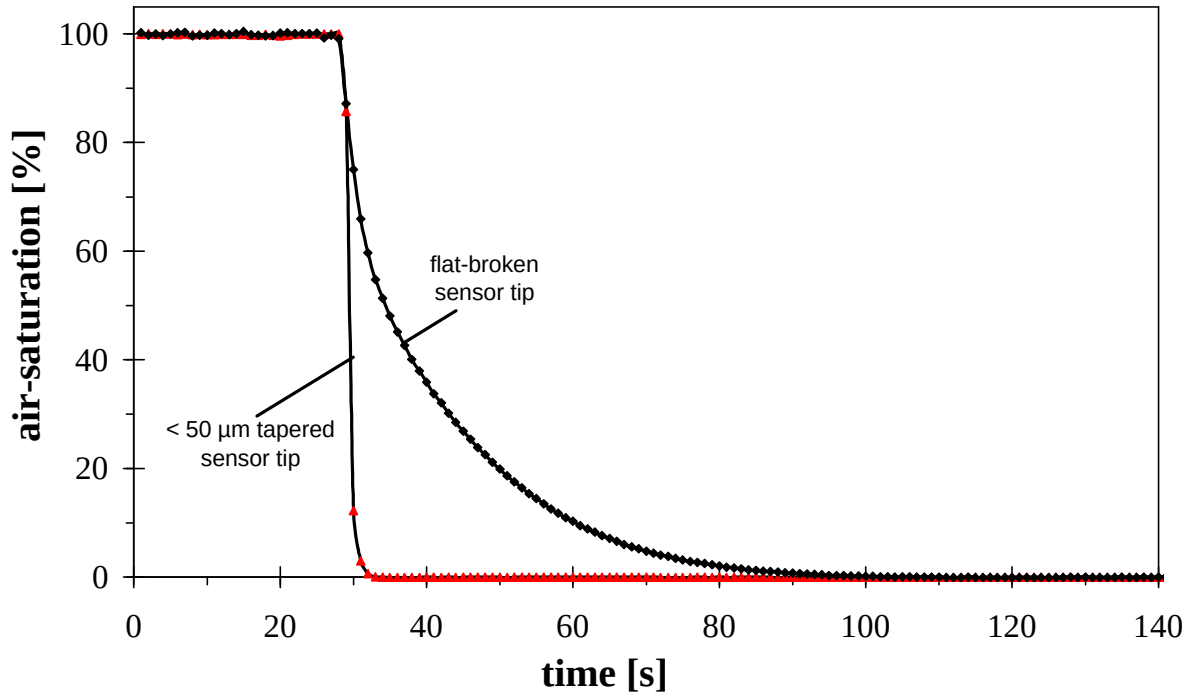


Figure 5.3 *Comparison of the response characteristics of a microsensor with a sensor tip tapered < 50 µm and a microsensor with a flat-broken sensor tip (Æ 140 µm).*

Sensor Stability

The oxygen-sensitive membrane stands sterilization by ethylene oxide, steam autoclavation (140 °C, 1.5 atm), CIP conditions (cleaning-in-place, 5% NaOH, 90°C), as well as a 3% H_2O_2 solution.

The oxygen-sensitive material may be subject to photo-decomposition resulting in a signal drift. Photo-decomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light.

Drift in % air-saturation at 100% air-saturation when illuminating the microsensor with a tapered and flat broken sensor tip for 1, 12 and 24 hours in the continuous mode (1 sec mode).

Mode	Drift per hour (3600 measuring points)	Drift per 12 hours (43200 measuring points)	Drift per 24 hours (86400 measuring points)
tapered sensor tip	< 0.6 % air-saturation	+1 % air-saturation	+1.6 % air-saturation
flat broken sensor tip	< 0.5 % air-saturation	< 0.5 % air-saturation	< 0.6 % air-saturation

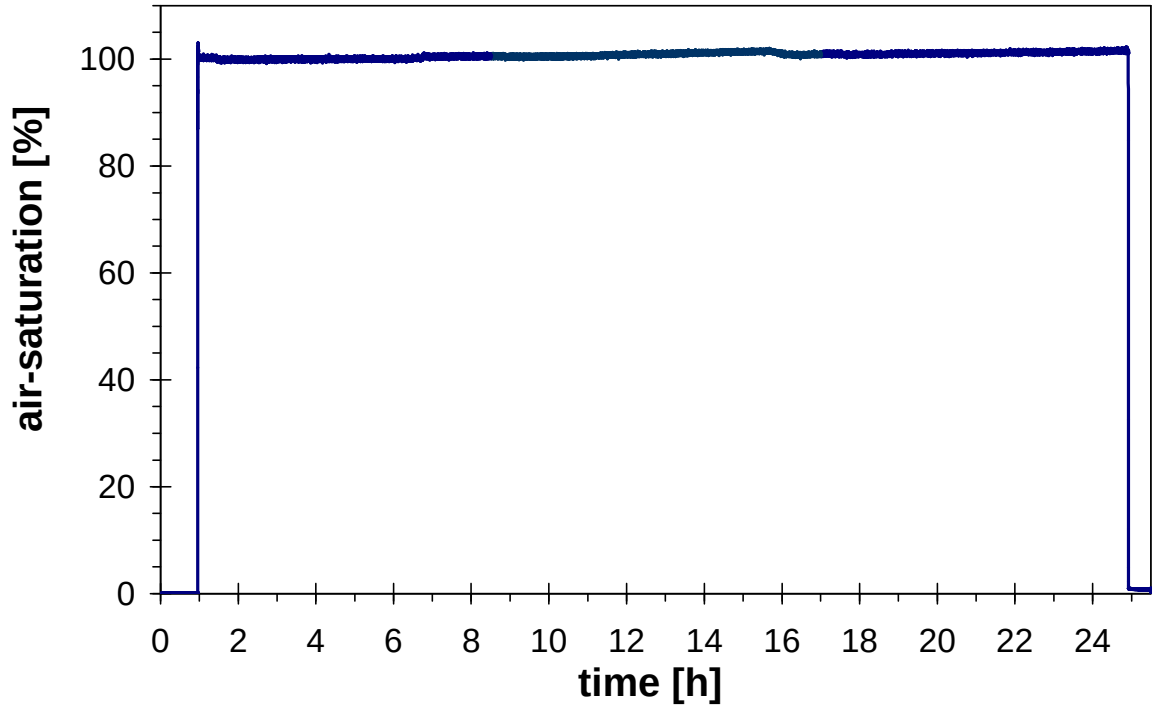


Figure 5.4 *Photostability of a tapered oxygen microsensor.*

5.1.3 Optical Isolation

Optical isolated sensor tips are required, if your sample shows intrinsic fluorescence between 600 - 700 nm. Consequently, an optical isolation is recommended measuring in whole blood, urine or chlorophyll containing samples. Using optical isolated sensors exclude the impact of colored samples and ambient light on measurements. Furthermore, an additional optical isolation improves chemical resistance of the sensor membranes.

Optical isolated sensor tips of oxygen microsensors enable measurement in photosynthetically active samples, since stimulation of photosynthesis, due to emission of blue-green light from the fiber tip, is avoided.

We offer additional optical isolation for all types of sensor. However, using optical-isolated sensor tips, the tip diameter increases to 60 - 80 µm, whereas tip diameter of non-isolated sensor tips is typically 30 to 40 µm for tapered microsensors. Since the response time of the microsensor is dependent from the diffusion rate of oxygen through the sensor layer, additional optical isolation, of course, increases the response time (t_{90}) to 3-7 seconds while the response time (t_{90}) of non isolated sensors is 1-3 seconds.

5.2 Housings of Oxygen-Sensitive Microsensors

The fiber-optic oxygen microsensors are based on 140 µm silica optical fibers. To protect the small glass fiber tip against breaking, suitable housings and tubings around it, depending on the respective application, were designed.

Of course, it is possible to build ***customer-specific*** housings. Please feel free to contact our service team to find the best solution for your application.

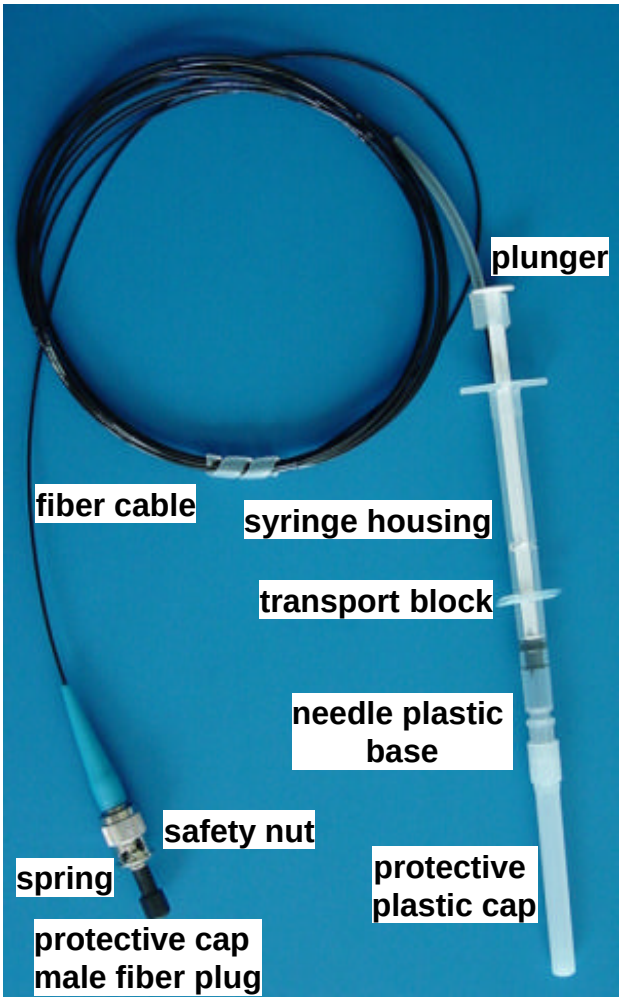
We offer the following standard designs:

Needle-type housing (**NTH**) oxygen microsensor.

Flow-through cell housing (**FTCH**) oxygen microsensor.

Implantable (**IMP**) oxygen microsensor.

5.2.1 Needle-Type Housing Oxygen Microsensors



We offer highly flexible needle-type oxygen microsensors. Needle-type oxygen microsensors are miniaturized optical chemical oxygen sensors designed for all research applications were a small tip size (< 50µm to 140 µm) and fast response time (t_{90} up to 1s) are necessary.

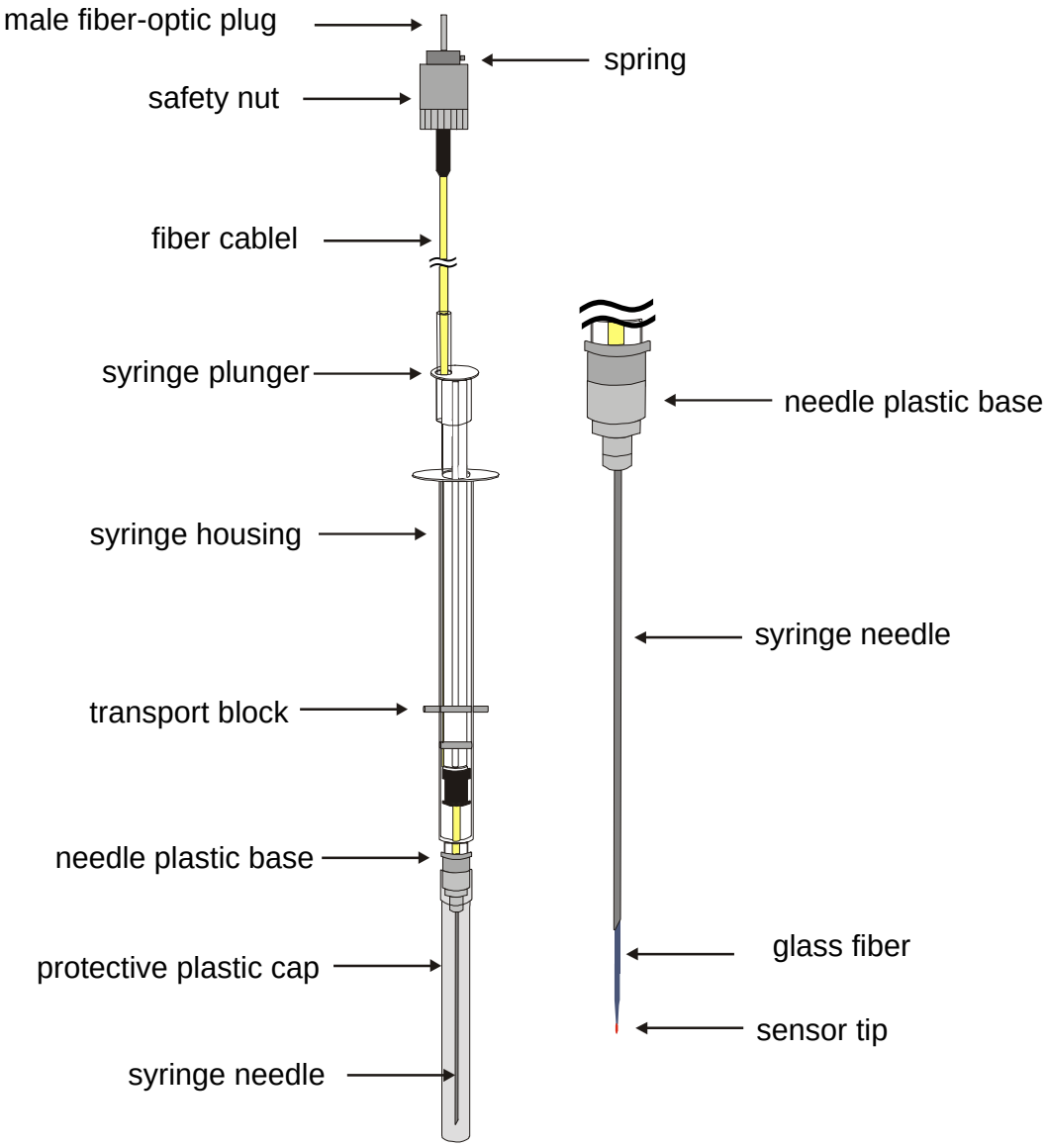
Needle-type oxygen microsensors are ideal for measuring oxygen distribution profiles in sediment and biofilms with a high spatial resolution of less than 50 µm. The glass-fiber with its oxygen-sensitive tip is protected inside a stainless steel needle and can be extended for measurement. If the sensor tip is sheltered inside this needle, it can be easily penetrated through a septum rubber or any other harsh material.

A 1 mL syringe tube made from polypropylene is used as the probe housing.

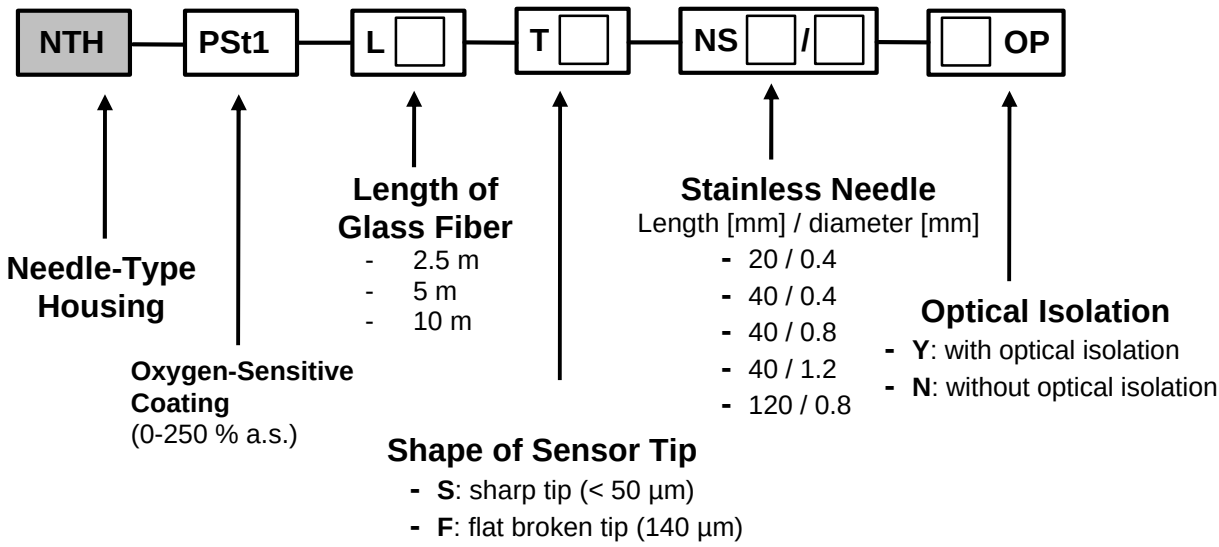
Features

- high spatial resolution
- penetration probe for insertion into semisolids like sediments or biofilms
- easy to handle and robust
- sterilizable (H_2O_2, EtO, EtOH)
- **not** autoclavable since the syringe is made out of polypropylene (For autoclavable needle-type probe housings please contact our service team)
- Measuring range from 0 to 250 % air-saturation (0-22.6 mg/L)
- Limit of detection 0.2 % air-sat, 20 µmol/L

Schematic drawing of a needle-type housing microsensor



Ordering information

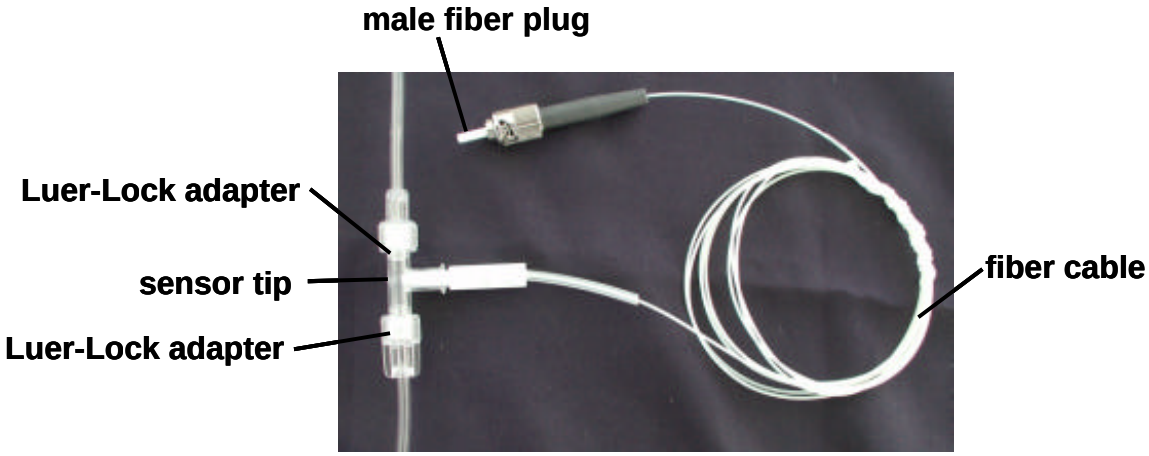


Example

With this code you will order a microsensor type **PSt1** mounted in a needle-type housing (***NTH***), with a glass fiber length of 5 m (***L5***), a sharp-tapered sensor tip of smaller than 50 µm (***TS***) mounted in a stainless needle of 40 mm length and 0.8 mm diameter (***NS 40/0.8***) containing the oxygen-sensitive fiber tip with additional isolation (***YOP***).

5.2.2 Flow-Through Cell Housed Oxygen Microsensors

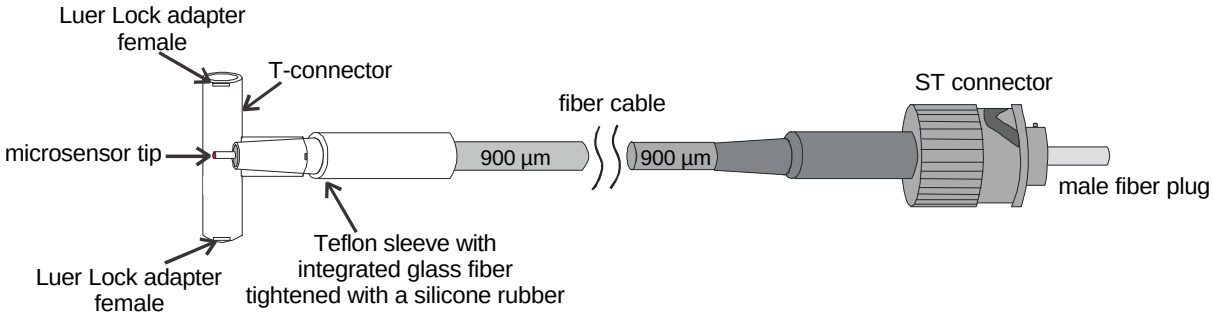
We offer miniaturized flow-through-cells with integrated oxygen microsensors. They can be connected via Luer-Lock adapters to tubings. Liquids (water, blood, ...) can be pumped through the cell. Online monitoring in real-time is possible.



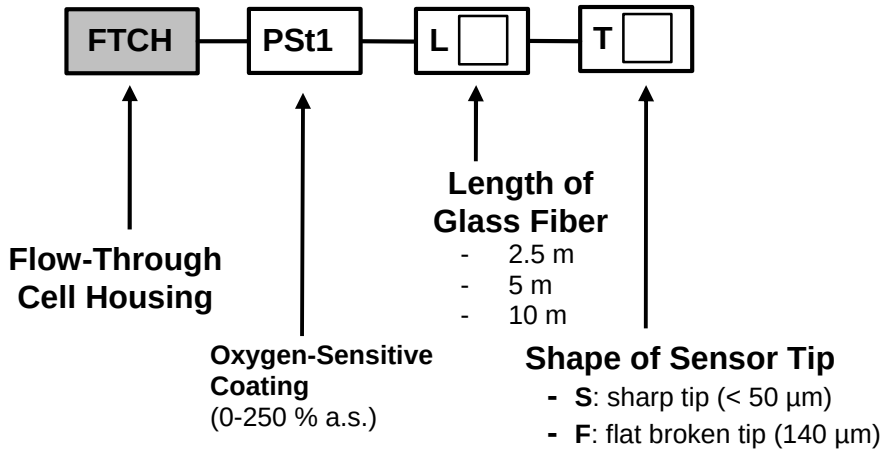
Features

- Easy to handle and robust
- Online monitoring
- Very fast response time
- Sterilizable (autoclave (130 °C, 1.5 atm), EtO, EtOH, H_2O_2)
- Measuring range from 0 to 250 % air-saturation (0-22.6 mg(L)
- Limit of detection 0.2 % air-sat, 20 µmol/L

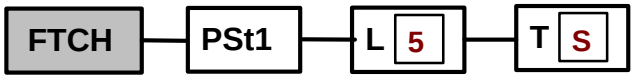
Schematic drawing of flow-through cell housed microsensors



Ordering Information

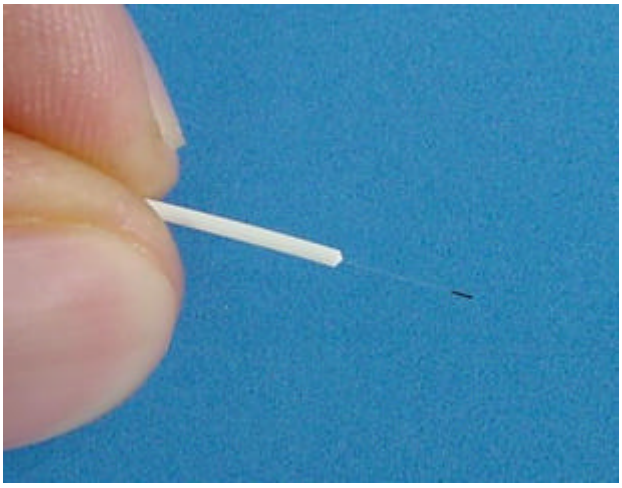


Example



With this code you will order a flow-through cell with (**FTCH**) an integrated microsensortype **PSt1**, with a glass fiber length of 5 m (***L5***), a sharp-tapered sensor tip of smaller than 50 µm (**TS**) containing the optical isolated oxygen-sensitive fiber tip.

5.2.3 Implantable Microsensors



We offer highly flexible implantable oxygen microsensors.

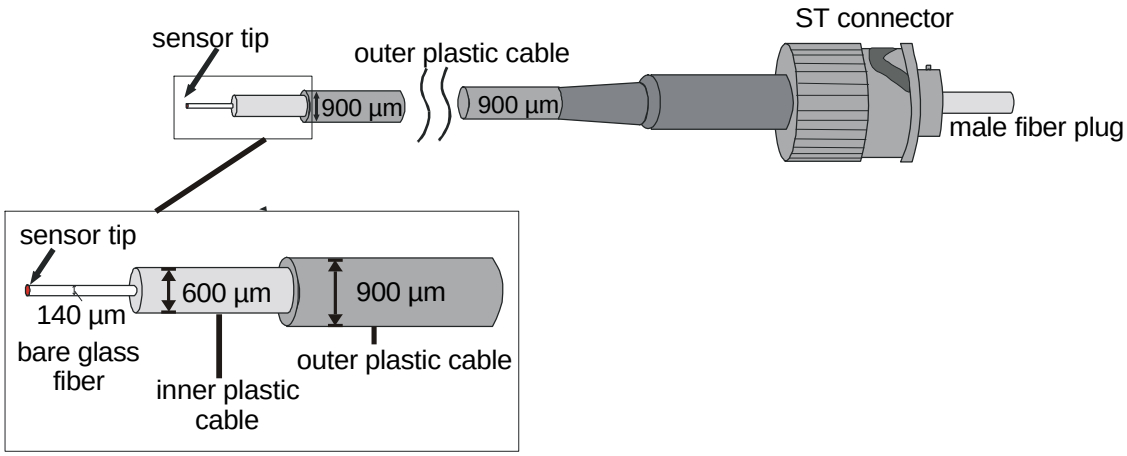
The microsensor tip is not housed in any additional housing. The bare glass-fiber tip can be mounted to your own costum-made housing, home-made steel tubes, costum-made micro respirometer chambers, etc. It can be deployed in soil or implanted into the blood circuits of living animals or the liquid circuits of trees to measure oxygen online and in real-time.

Small outer diameters of 900 or even 600 µm allows insertion into implanted Venflon-tubes.

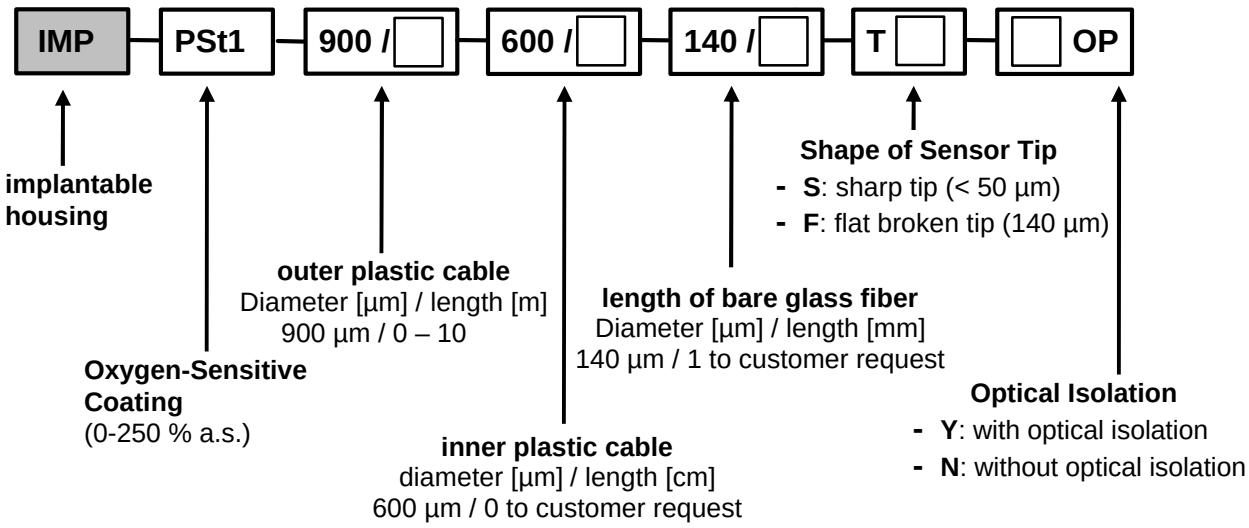
Features:

- High spatial resolution
- High flexibility
- Without any housings (the microsensor is protected with a glass housing during the transport)
- Sterilizable (autoclave (130 °C, 1.5 atm), EtO, EtOH, H_2O_2)
- Implantation into animal blood circuits
- Soil implantation
- Implantation in customer-made housings
- Measuring range from 0 to 250 % air-saturation (0-22.6 mg(L)
- Limit of detection 0.2 % air-sat, 20 µmol/L

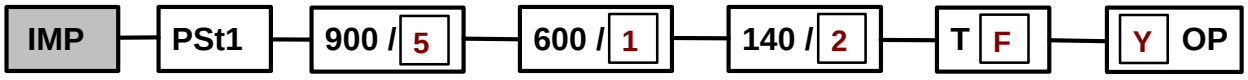
Schematic drawing of implantable microsensors



Ordering Information



Example



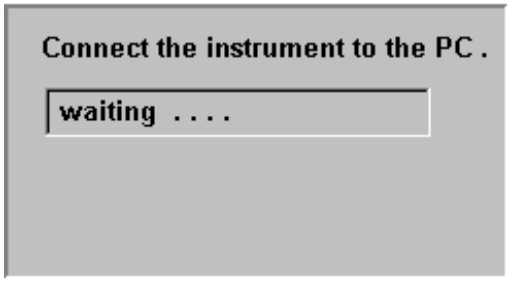
With this code you will order an implantable (***IMP***) microsensor type ***PSt1*** mounted in a glass housing. The outer plastic cable with a diameter of 900 µm is 5 m long (***900 / 5***), the inner plastic coating (∅ 600 µm) 1 cm (***600 / 1***) and a bare glass fiber length (∅ 140 µm) of 2 mm (***140 / 2***). The sensor tip that is flat broken (***TF***) containing the oxygen-sensitive fiber tip with additional optical isolation (***YOP***).

6 Description of Microx TX3 Software

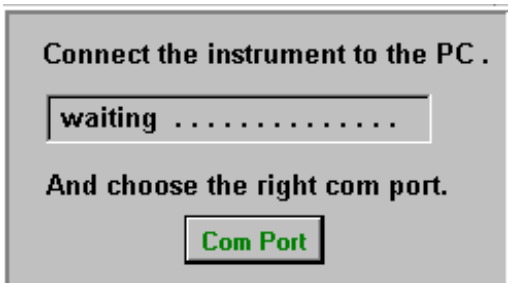
This software is compatible with Windows 95/98/2000/Millenium/NT4.0/XP.

6.1 Software Installation and Starting the Instrument

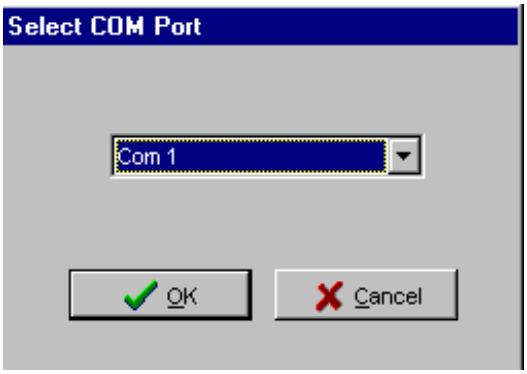
1. Insert the supplied disc/CD into the respective drive. Copy the file TX3v531.exe onto your hard disk. (for example, create C:\MICROXTX\TX3v531.exe). Additionally, you may create a link (Icon) on your desktop.
2. Connect the Microx TX3 via the supplied serial cable to a serial port of your computer. Tighten the cable with the screws on your computer and on the Microx TX3.
3. Connect the power supply.
4. Please close all other applications as they may interfere with the software. Start the program TX3v531.exe with a double click. The following information window appears:



5. If the right com port is adjusted this information window disappears within a few seconds. If the wrong com port is adjusted you are asked to set the right com port:



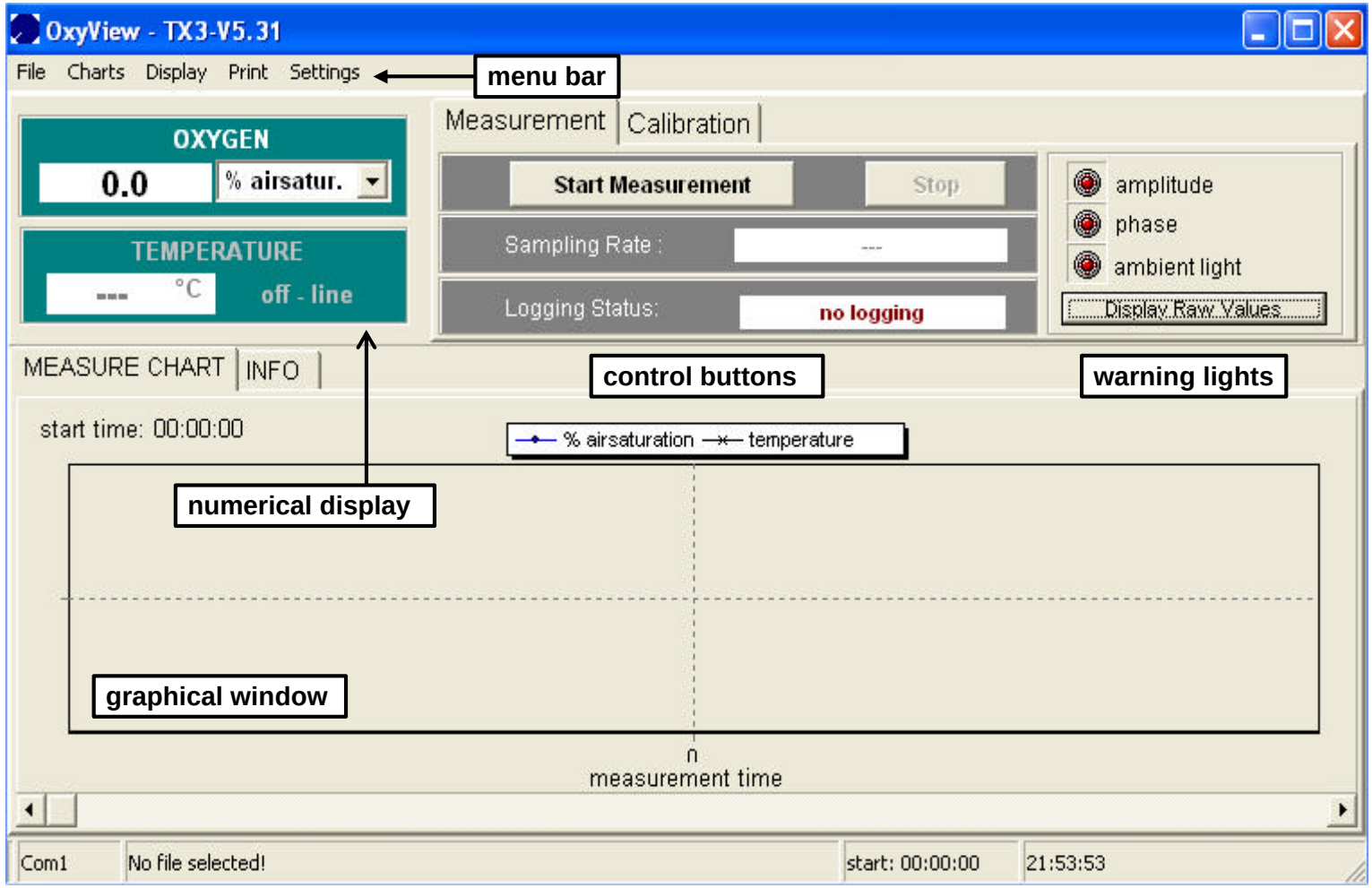
With a right mouse click onto '***com port***' you are able to set the right com port. Please confirm your selection by clicking the '***OK***' button. The information window disappears if the right com port is adjusted.



6.2 Function and Description of the Microx TX3 Program

The window shown below is displayed after starting the software microx.exe:
The program has 4 main sections:

1. Menu bar
2. Graphical window
3. Status bar
4. Control bar, divided into numerical display, control buttons and warning lights



6.2.1 Menu Bar

File	*Charts*	*Display*
→ Exit	→ Oxygen	→ Zoom → AutoScaleY1 → Undo Zoom
	→ Phase	
	→ Amplitude	→ Clear Charts
	→ Temperature	→ Dimensions

Print	*Settings*
→ Charts	→ Com Port
	→ Instrument Info
	→ analog settings
	→ LED Intensity

File

Exit

Closes the program.

Charts

The respective charts of the measurement can be displayed (***v***) or hidden

Oxygen:
Oxygen content in the chosen unit

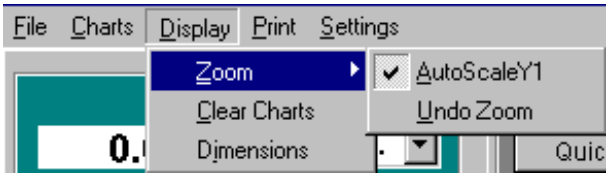
Phase:
Phase angle, the raw data

Amplitude:
The magnitude of the sensor signal

Temperature:
The measured temperature

Display

Zoom:

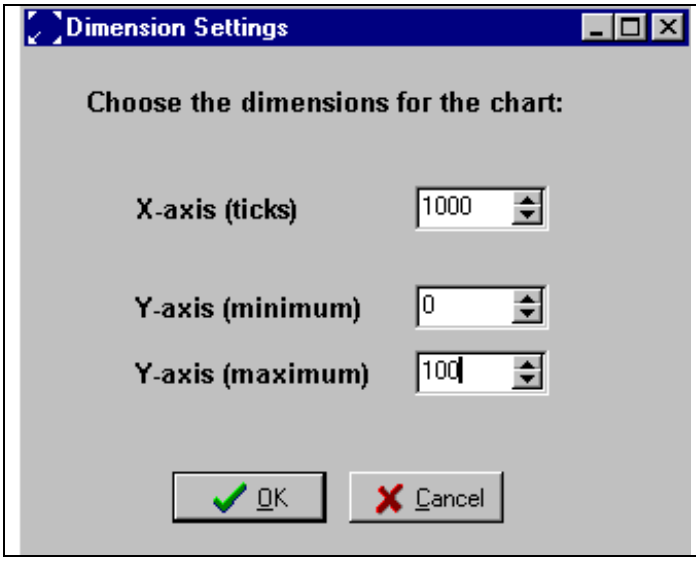


AutoScaleY1 is the default setting. AutoScaleY1 means that the y-axis is scaled automatically.

Undo Zoom: The original display is recovered; see also graphical display

Clear Charts: The graphs shown on the display is cleared.

Dimensions:



You can adjust the number of measurements points on the x-axis shown in the display (maximum number of points are 5000)

Furthermore, you can adjust the minimum and the maximum of the y-axis.

The AutoScaleY1 function is switched off.

Print

Charts: *The charts shown in the display can be printed*

Settings

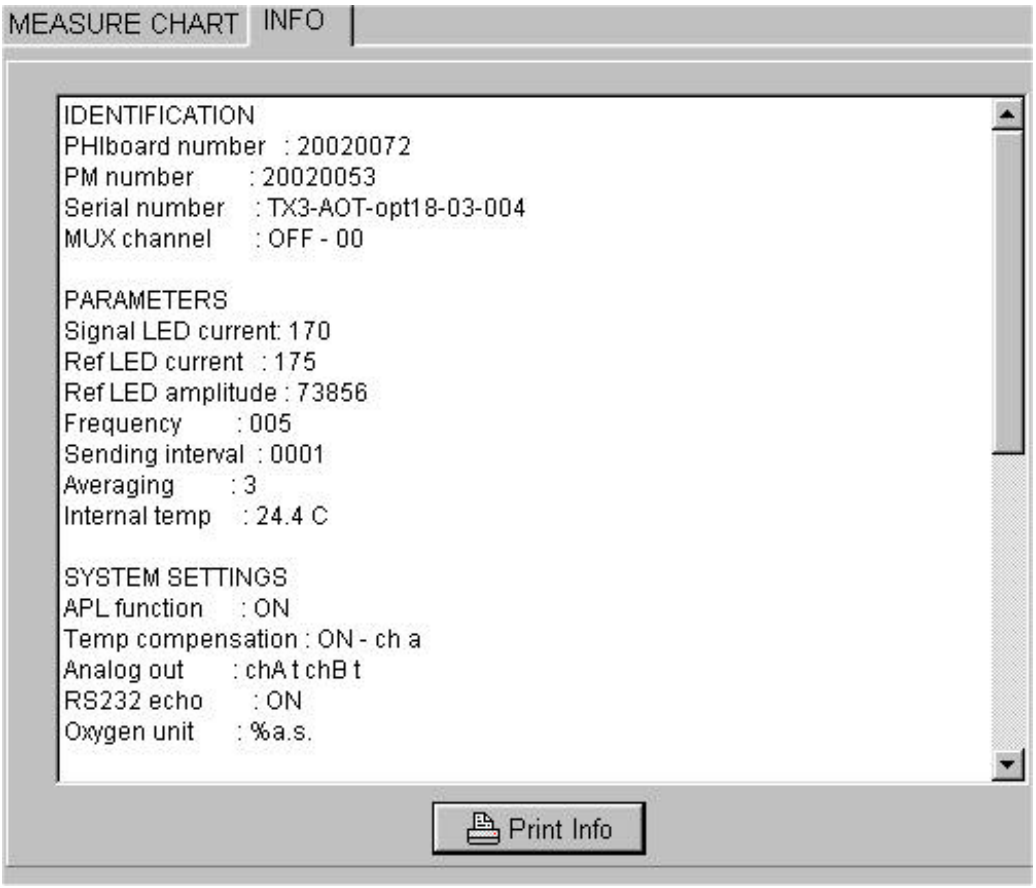
ComPort

The serial comport (com1 – com20) for the serial interface (RS 232) can be chosen in this window. COM 1 is the default setting. If you choose the wrong Com port, the information window '***Connect the instrument to the PC and choose the right com-port***' does not disappear.

Instrument Info:

Here you can find the version of the software and some important settings of the instrument. If you have a problem with the Microx TX3 oxygen meter, please contact our service team and have the software and instrument information ready.
To change back to the graphical window click the '***Measure Chart***' button.

Instrument Info



Software Info

LED-Intensity

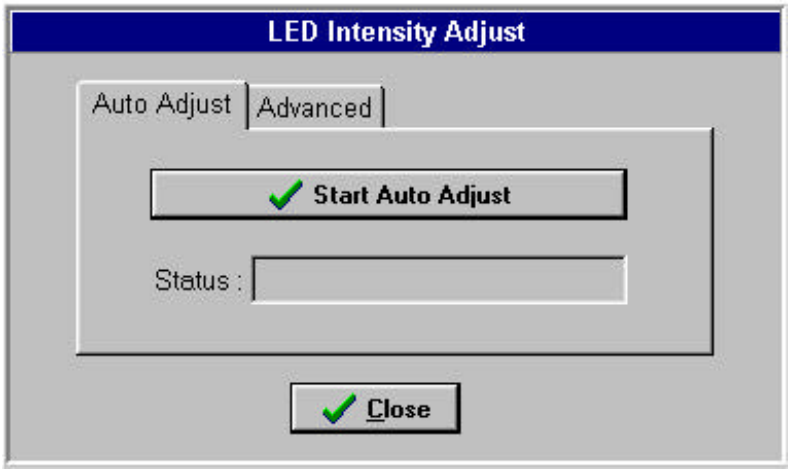
With the current of the LED you can adjust the amount of light illuminating the sensor spot.

You can choose between an '**Auto Adjust**' of the LED where the Microx TX3 adjusts the optimal LED current itself, or you can select '**Advanced**' where you can adjust the LED current yourself.

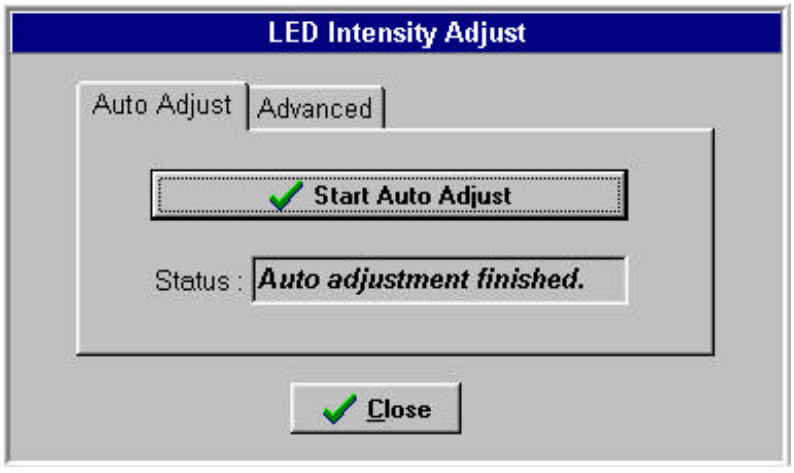
If you increase the LED current, the signal amplitude increases, since a higher light density illuminates the sensor spot.

Auto Adjust:

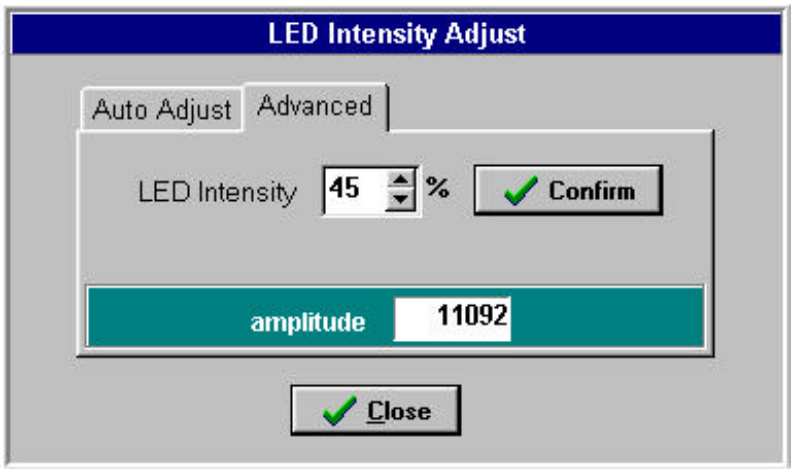
To make the adjustment of the LED intensity automatically, just click the button '**Start Auto Adjust**'. Please check that the oxygen microsensors has been connected to the instrument.



The automatically adjustment of the LED intensity is finished when in the status window the message '**Auto adjustment finished**' appears. Click the '**Close**' button to confirm the settings.



Advanced:
Click the '**Advanced**' button to change the LED current manually. Values between 10 and 100 % are possible. After clicking the 'confirm' button you can see the change of the amplitude in the window below.



Please note, that after changing the LED intensity you should re-calibrate the oxygen microsensor. A warning window points you to re-calibrate the oxygen microsensor.

LED Adjust - Warning
The calibration values are no longer valid.
Please recalibrate the sensor.
Calibration
I will recalibrate later.

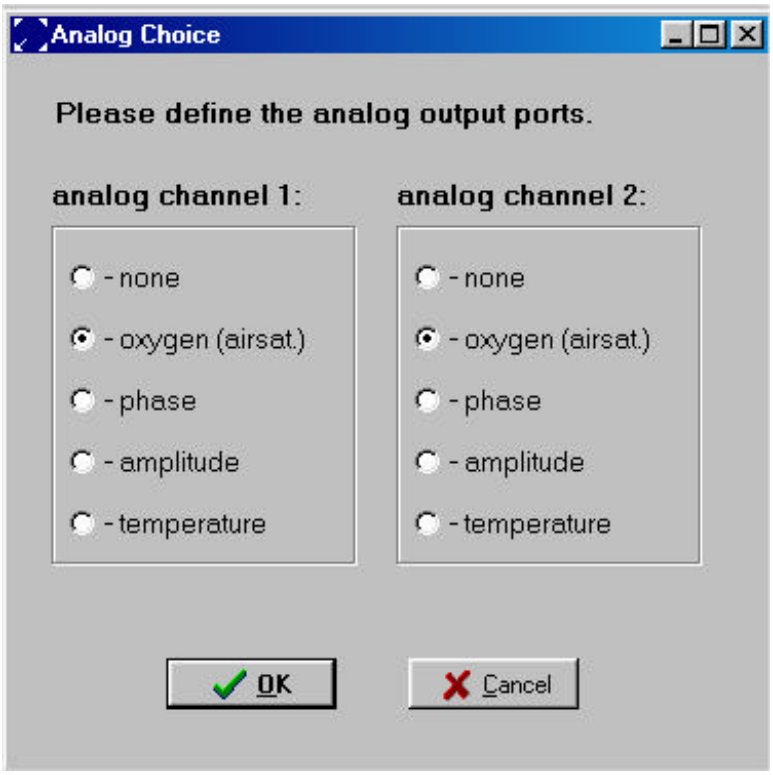
Please note:

By increasing the light intensity you increase the amplitude of the oxygen microsensor. This leads to smoother phase signals. However, increasing the light intensity can increase photobleaching, which decreases the shelf-life of your sensor.

Analogue output
Here you can choose which data are exported via the analog output. The
Microx TX3 device has two analog outputs and one trigger input. The desired data sources (oxygen, temperature, amplitude, phase) can be chosen via the dialog box.

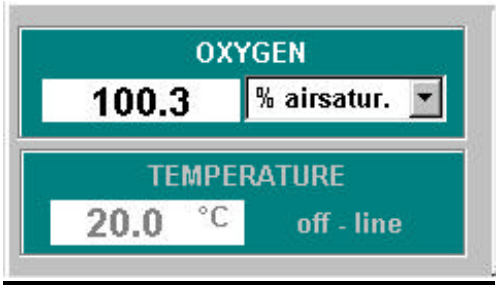
Equivalence coefficient

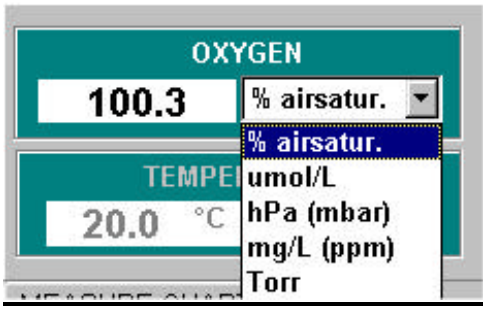
oxygen	1 : 0.1 (e.g. 973mV = 97.3 % air saturation)
temperature	1 : 0.1 (e.g. 208mV = 20.8°C)
amplitude	1 : 20 (e.g. 1110mV = 22200 relative units)
phase	1 : 0.025 (e.g. 1100mV = 27.50°)



6.2.2 Control Bar

Numerical display





The actual oxygen content in the chosen unit (here % air-saturation) is displayed in the oxygen window. The oxygen unit can be changed by clicking the pull down menu. Tables and formulas for the calculation of different concentration scales are given in the appendix.

Please note:
It is also possible to change the oxygen unit during the measurement.

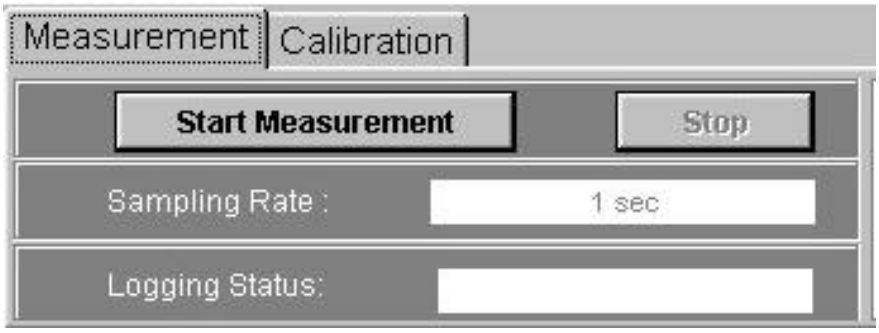
Temperature measurement:
The actual temperature value of the sample (in the case of temperature compensated measurements) is displayed in the temperature window.
If measurement is performed without temperature compensation, the manual inserted temperature is displayed with the hint that temperature measurement is off–line.

Control buttons:

The way to start a measurement is

(A) Calibration of the minisensor with the Calibration Assistant

(B) Start Measurement with Measurement Assistant

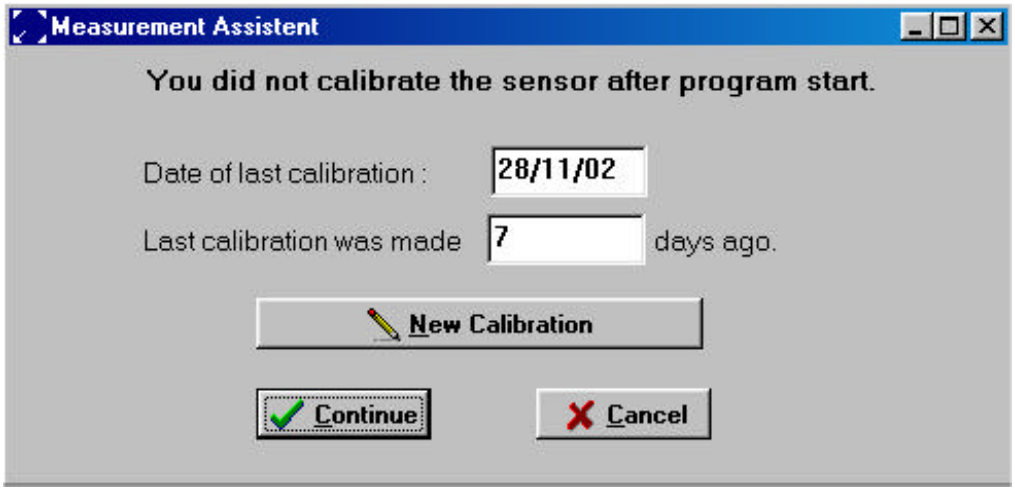


(A) Calibration:

The calibration assistant is opened (see chapter 7, Calibration of Oxygen-Sensitive Microsensors);

(B) Measurement:

By clicking '***Start Measurement***' the measurement assistant opens to enter the measurement settings.
If you haven't performed sensor calibration yet the following window appears



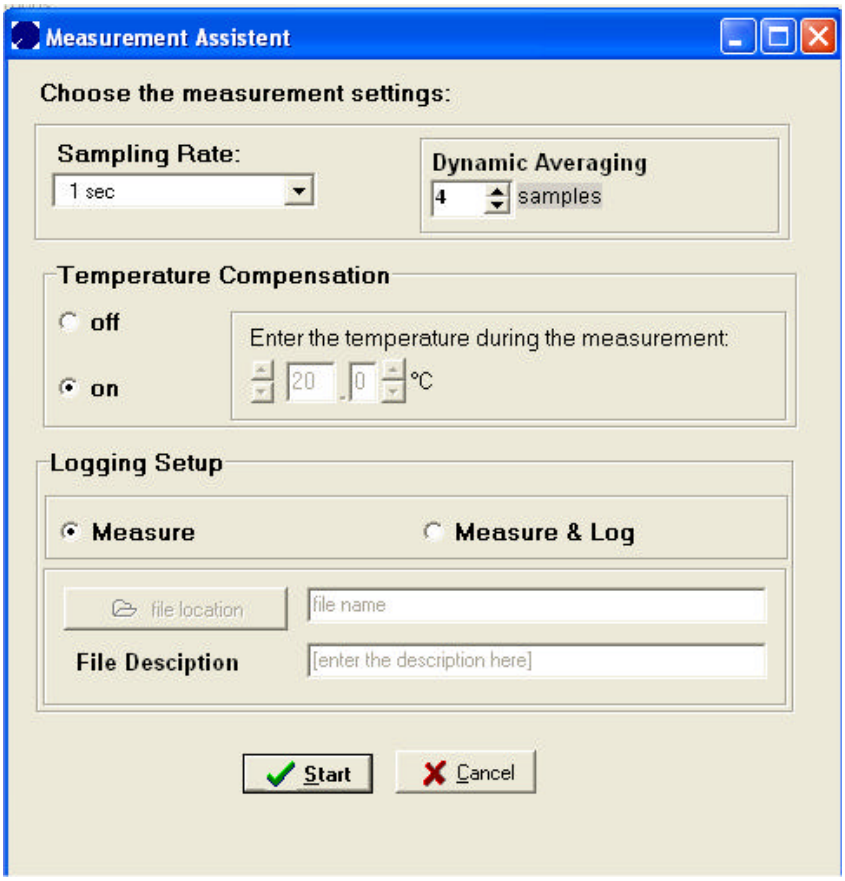
If you want to measure with the last sensor calibration - you can find the '***date of the last calibration***' in the window - click the '***Continue***' button. To obtain reliable results we strongly recommend to perform a sensor calibration before measurement by clicking the '***New calibration***' item when connecting a new sensor.

Follow the instructions given in chapter 7 to calibrate the respective microsensor.

To leave this menu, click the '***Cancel***' button.

If you have already performed sensor calibration, the measuring assistant will be opened.

In this window you can choose the measurement settings:



In the '**Sampling Rate**' window you can select the desired measurement mode with a drop-down menu.

By clicking the drop down menu you can choose from '***fast sampling***' (update rate each 250 – 350 ms) to the '***60 min***' mode where each hour a measuring point is recorded.

The speed of recording a measurement point in the '***fast sampling***' mode is about 250 ms when no temperature sensor is connected and decreases to about 350 ms when connecting a temperature sensor or activating the analog output channels.

Please note:

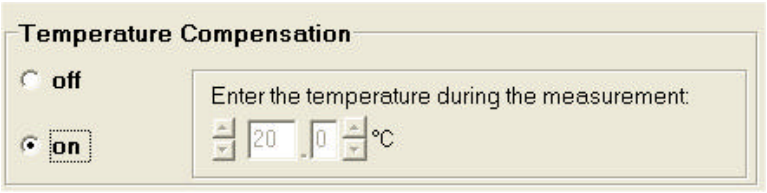
The sensor shelf life can be increased using a slower measuring mode since the effect of photo-bleaching is reduced. The illumination light is switched off between sampling. A further advantage using a high measuring mode is that huge amounts of data for long-time measurement can be avoided.

Dynamic averaging

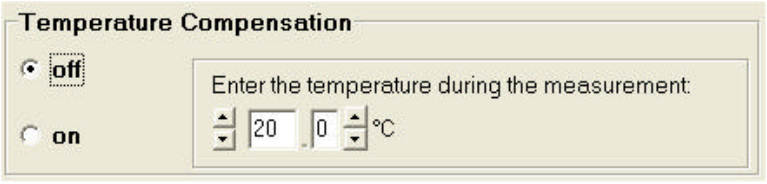
The '***dynamic average***' defines number of averaged measured values. The higher the running average, the longer the time (sampling time) used for averaging. The higher the running average is set, the smoother the measurement signal (maximum 25 samples); The default setting is 4.

Temperature compensated oxygen measurements

If you want to measure with temperature compensation, click the '***on***' button. Please ensure that the temperature sensor Pt 1000 is connected to the Microx TX3, before you click the '***Start***' button to continue. The window where you can enter the temperature manually is disabled.

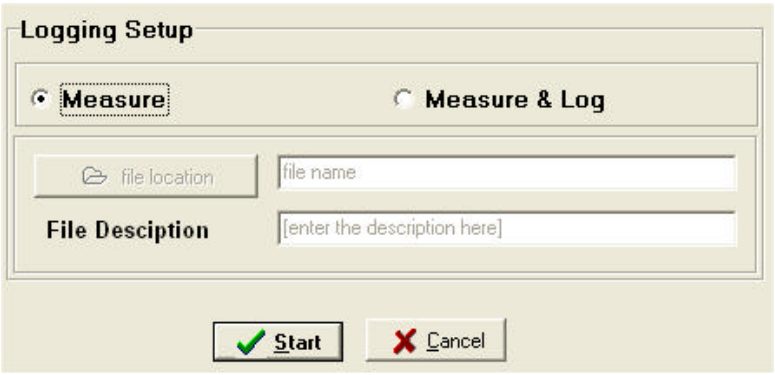


If you want to measure without temperature compensation, choose the '***off***' button. Please enter the temperature of your measurement sample manually. Click the '***Start***' button to start the measurement.

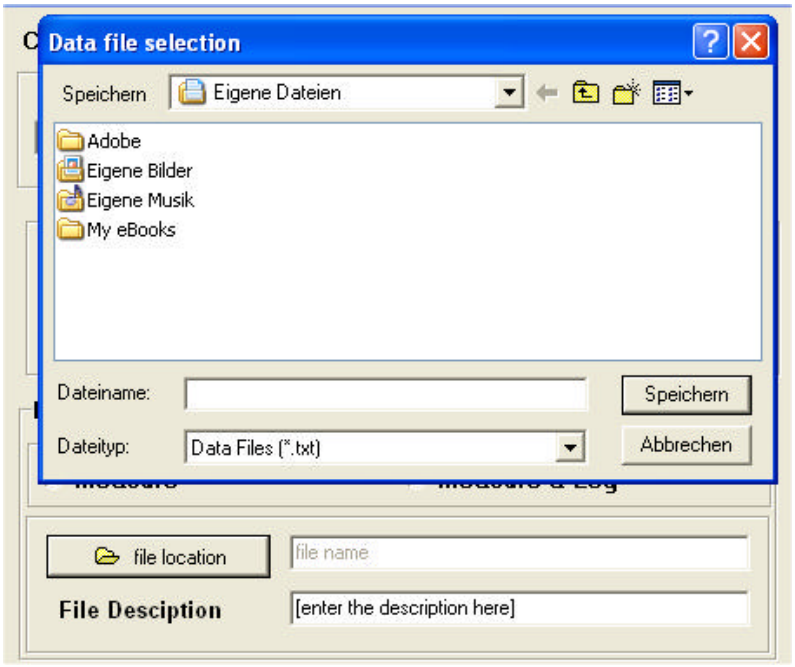


Logging Setup:

To strat the measurement without logging data click '***Measure***' in the Logging setup and the '***Start***' button.

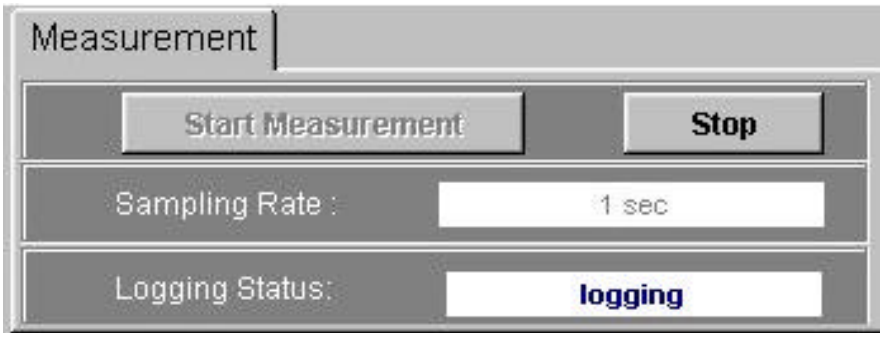


To store the data of your measurement click in the '***Logging Setup***' the '**Measure & Log**' item and click the button '***Choose File***'. Here you can select the location where you want to store the data. Choose as file extension ****.txt***. Click the '***speichern***' button to confirm your settings.



You can enter a measurement description in the text field '***File description***' which is stored in the Ascii File. To start the measurement, click the '***Start***' button.

In the Information windows '***Sampling Rate***' you can find the adjusted sampling rate. To be sure whether you perform a measurement with or without logging the data, the '***Logging Status***' window displays whether the actual measurement is stored to a file (***logging***) or not (***no logging***);

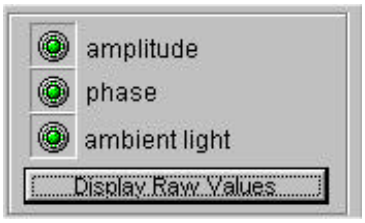


Stop Measurement

The measurement is ended by a left click on the '***stop***' button in the control bar.

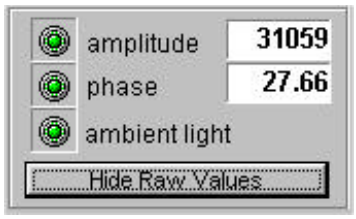
Warning Lights:

At the right bottom of the window you can find the amplitude, phase angle and three warning lights. The warning lights are explained below:



amplitude:	red:	Amplitude is too low, the sensor tip may be damaged or sensor cable may not be connected
	yellow:	Amplitude is critically low, replacement of the sensor is recommended
	green:	amplitude is correct
phase:	red:	phase angle is out of limits
	green:	phase angle is in normal range
ambient light:	red:	background light (e.g. direct sunlight, lamp) is too high. Decrease of false light is recommended
	green:	ratio of sensor signal to false light is acceptable

By clicking the 'Display Raw Values' button, the raw data of phase angle and amplitude are displayed next to the warning lights.



6.2.3 Graphical Window

The respective sensor signal is displayed according to the selection of the 4 control buttons oxygen, phase, amplitude and temperature (menu chart). The oxygen content is displayed in the chosen unit, the temperature in °C. The raw values (the phase angle in degrees and the sensor amplitude in mV) can also be displayed by clicking the button ***'Display Raw values'***.

Zoom Function:

1. Press the left mouse button and drag from left to right to enlarge a certain area of the graphical window. The graphical window displays the selected data points and is not actualized with new data.
2. Press the left mouse button and drag from right to left to recover the original display, or click the '**Undo Zoom**' button in the *display* menu under *zoom*.

6.2.4 Status Bar

sw1: Displays the serial port which is used for communication of the Microx TX3 device with the PC
sw2: Displays the file name in which the measurement data are stored. „No storage file selected“ is displayed if no file was selected (no data storage).
sw3: Displays the start time of the measurement
sw4: Displays the actual time

6.3 Subsequent Data Handling

In the head of the ASCII file, you find the **description** of your measurement which you have entered by storing the file.
Below you find the ‘***instrument info***’ containing the data of the complete calibration routine and some more important settings of the instrument and firmware.
The ‘***software info***’ below contains the version number of the Microx TX3 software, date and time of the performed measurement. If there is a problem with the Microx TX3 oxygen meter, please contact our service team and have the software and instrument information ready.
Below, you find the ‘***measure mode settings***’ containing the *dynamic averaging*, and the *measuring mode*.

The following rows, separated by semicolons, list the measuring data. The first two rows contain the **date** and **time**, the third the **log-time** in minutes, the fourth the **oxygen content** in the chosen unit. The raw data - **phase angle** in [°] and the **amplitude** in [mV] - are stored in the fifth and sixth row, respectively. The seventh row contains the **temperature** in °C measured by PT100 temperature sensor. Raw data can be used for user defined recalculations according to the formulas and tables listed in the appendix.

***** DESCRIPTION **********
test measurement

***** INSTRUMENT INFO *******
***
IDENTIFICATION
PHIboard number : 20020069
PM number : 20020032
Serial number : TX3-AOT-2003-0001---
MUX channel : ON - 01

PARAMETERS
Signal LED current: 160
Ref LED current : 075
Ref LED amplitude : 106091
Frequency : 005
Sending interval : 0001
Averaging : 1

SYSTEM SETTINGS
APL function : ON
Temp compensation : OFF
Analog out : chA o chB o
RS232 echo : ON
Data logger : OFF - 0/800
Oxygen unit : %a.s.

CALIBRATION
Sensor type : 1
0% a.s. phase 1 : 56.00 at 21.0°C amp 000000
100% a.s. phase 2 : 28.07 at 20.0°C amp 021100
Date (ddmmyy) : 060103
Pressure (mBar) : 1013

FIRMWARE
Code ver 1.077 : 12/12/02, 12:50:57
Xilinx built : 20/08/02 (MM/DD/YY)
Reset condition : CONTINUOUS

***** SOFTWARE INFO *********
OxyView - TX3-B2-V5.00 12/2002
© by PreSens
06.01.03
13:12:08

******MEASURE MODE SETTINGS**
Dynamic Aver 1
measure mod 1 sec

start time 12:50:01

date	time/hh:mm:ss	logtime/min	oxygen/% airsatur.	phase/°	amp	temp/°C
06.01.03	13:12:09	0.000	101.510	27.90	20808	20.00
06.01.03	13:12:10	0.017	101.410	27.91	20797	20.00
06.01.03	13:12:11	0.034	101.370	27.92	20785	20.00
06.01.03	13:12:12	0.051	101.960	27.85	20776	20.00
06.01.03	13:12:13	0.069	101.640	27.89	20776	20.00
06.01.03	13:12:14	0.085	101.530	27.90	20761	20.00
06.01.03	13:12:15	0.102	101.710	27.88	20774	20.00
06.01.03	13:12:16	0.119	101.730	27.88	20754	20.00
06.01.03	13:12:17	0.136	101.710	27.88	20747	20.00
06.01.03	13:12:18	0.154	102.080	27.84	20738	20.00
06.01.03	13:12:19	0.170	101.460	27.91	20739	20.00
06.01.03	13:12:20	0.188	101.790	27.87	20736	20.00
06.01.03	13:12:21	0.205	101.760	27.88	20717	20.00
06.01.03	13:12:22	0.222	101.920	27.86	20738	20.00
06.01.03	13:12:23	0.239	101.820	27.87	20718	20.00
06.01.03	13:12:24	0.256	101.990	27.85	20714	20.00
06.01.03	13:12:25	0.273	102.560	27.79	20711	20.00

7 Calibration of Oxygen-Sensitive Microsensors

7.1 Calibration of Needle-Type Oxygen Microsensors

7.1.1 Preparation of the Calibration Standards

Calibration of microsensors is performed using a conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***water-vapor saturated air*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add one gram sodium sulfite (Na_2SO_3) to the vessel and label it '**cal 0**';
2. Dissolve Na_2SO_3 in 100 mL water.
   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination

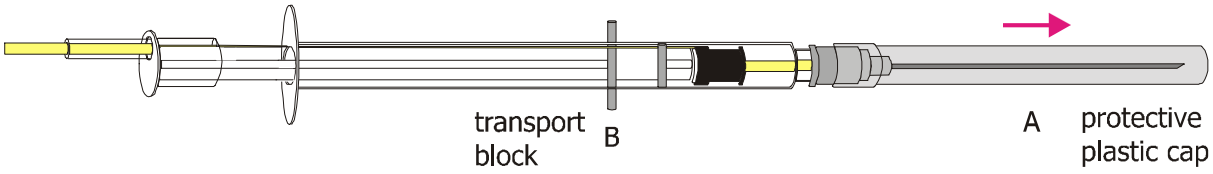
To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of '**cal 0**' is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration standard 100 (water-vapor saturated air)

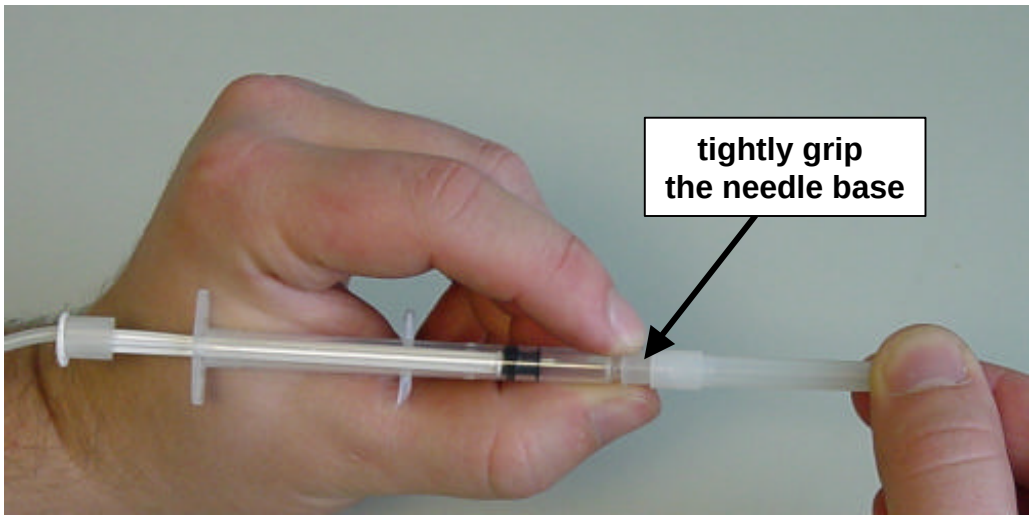
1. Place wet cotton wool in the vessel with the label '**cal 100**'.
2. Drill two holes for inserting the microsensor and the temperature sensor in the screw top and close the vessel.
3. Wait about 2 minutes to ensure that air is water-vapor saturated

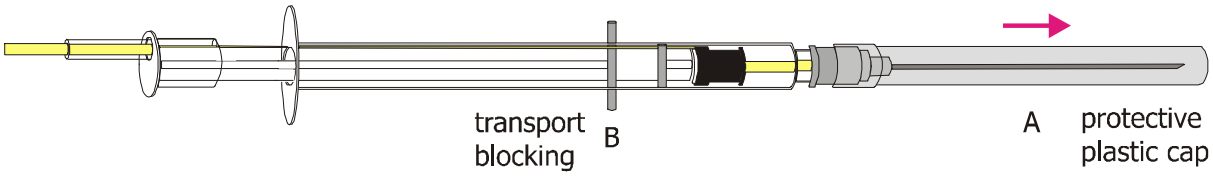
7.1.2 Mounting the Needle-Type Microsensors

1. Remove the microsensor carefully from the protective cover. The needle-type microsensor is housed in 0.4 x 40 mm syringe needle mounted to a 1 mL plastic syringe housing with integrated PUSH & PULL - IN & OUT mechanism. The syringe needle is protected with a protective plastic cap (**A**).

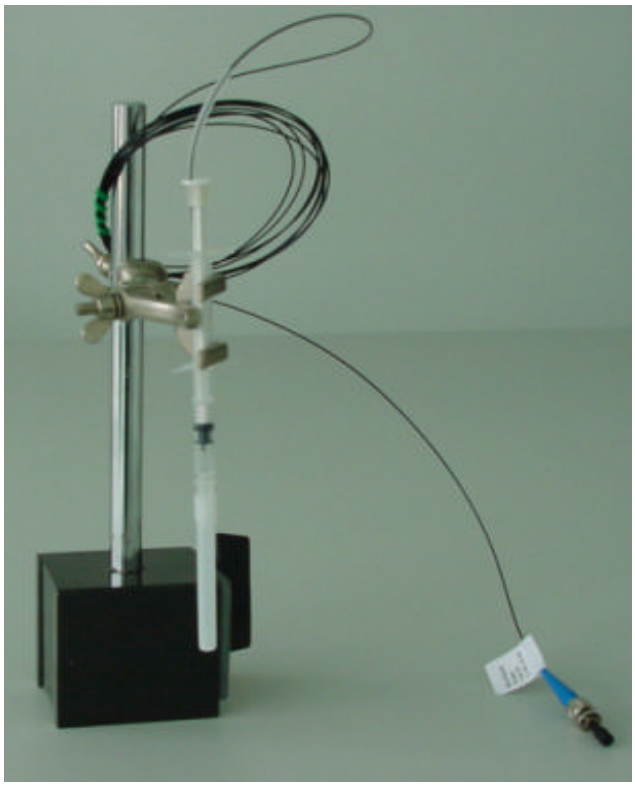


2. Carefully remove the protective plastic cap (**A**) covering the syringe needle.
When doing so, grip the plastic base of the needle tightly. The syringe needle ***must not*** be removed from the syringe housing. Work carefully!



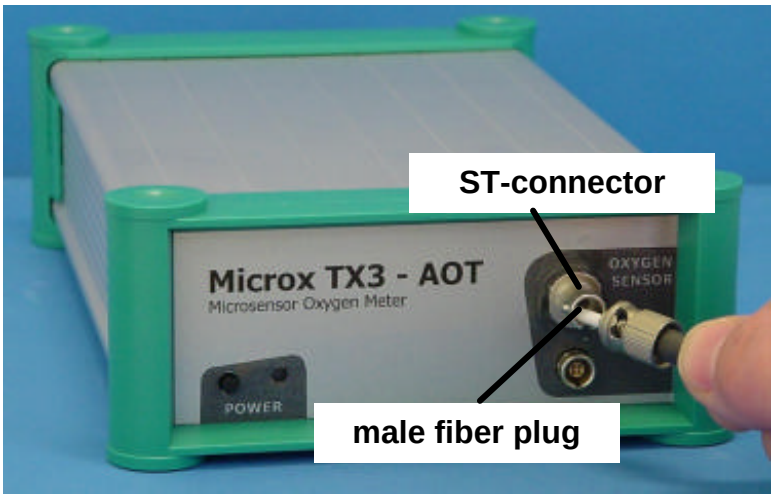


3. Fix the microsensor with a clip to a laboratory support or a similar stable construction.



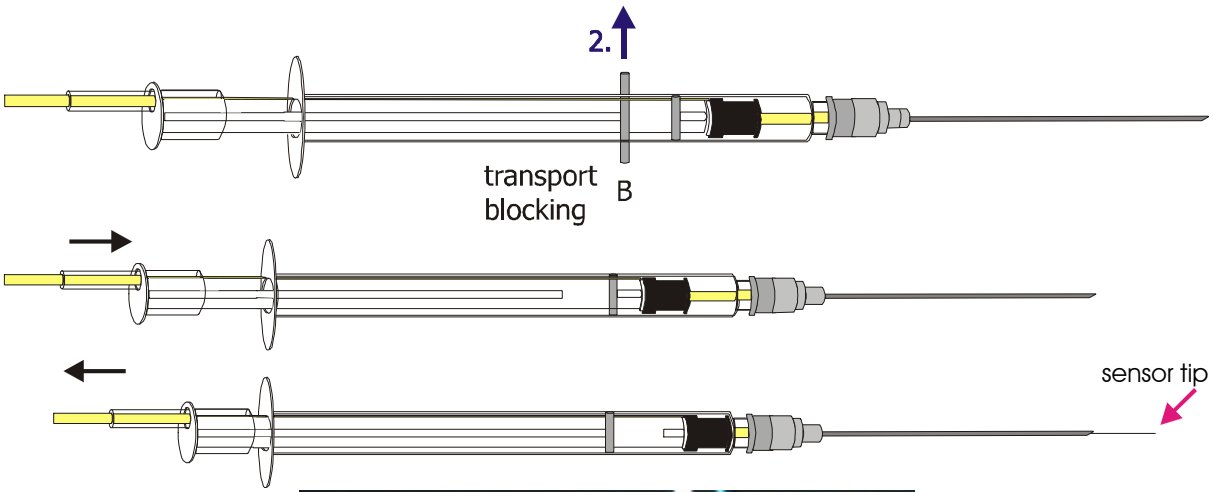
We expressly warn you not to handle with microsensors without the support - especially when the sensor tip is extended.

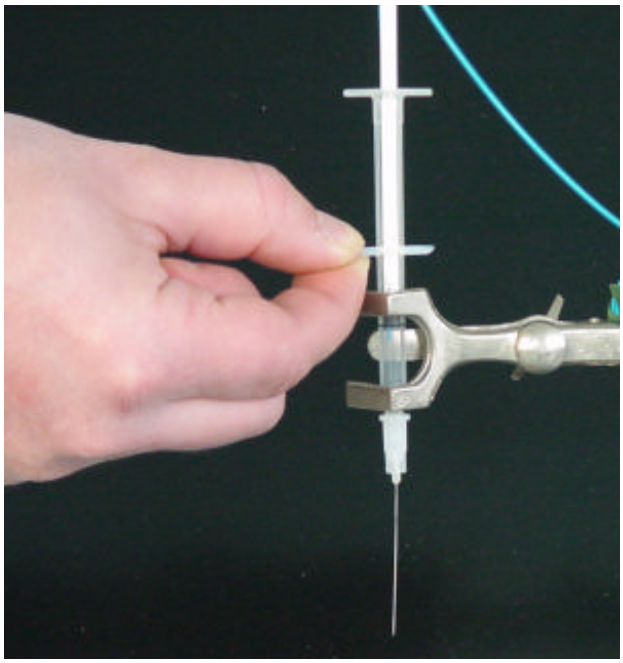
4. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the Microx TX3 device. The female fiber-plug of the Microx TX3 has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.



5. The glass fiber with its sensing tip is prevented from slipping using a transport block (**B**). Remove the transport block from the hole in the syringe housing. Now it is possible to retract or extend the glass fiber with its sensor tip by pushing or pulling the plunger.

Before pushing out the sensor tip, make sure that you have removed the protective plastic cap and have some space in front of the syringe needle.





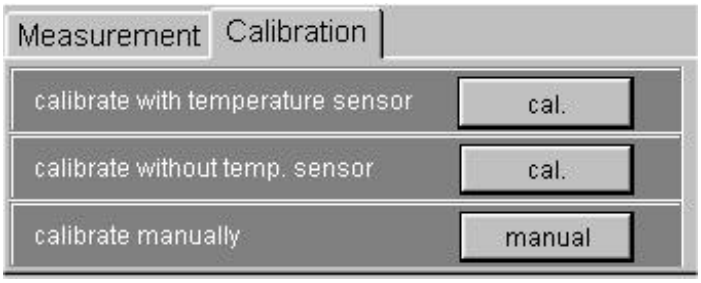
WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK

7.1.3 Calibration without Automatic Temperature Compensation

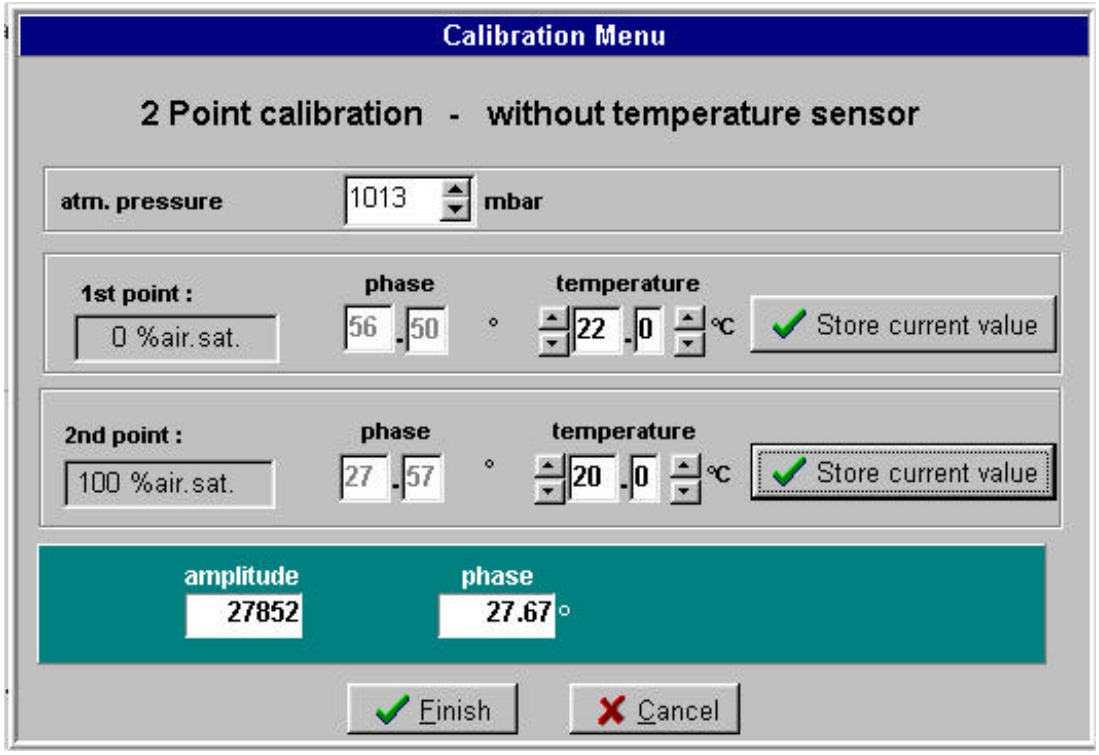
Using the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor PT 1000 is not connected to the Microx TX3.

Please note:
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen microsensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. Switch on the Microx TX3 and connect the microsensor as shown in Chapter 7.1.2 '***Mounting the Needle-Type Microsensors***'.
3. Start the Microx TX3 software on your computer and click the '***calibration***' menu item.
4. Select the calibration routine: '***calibrate without temp sensor***' and click the '***cal.***' button

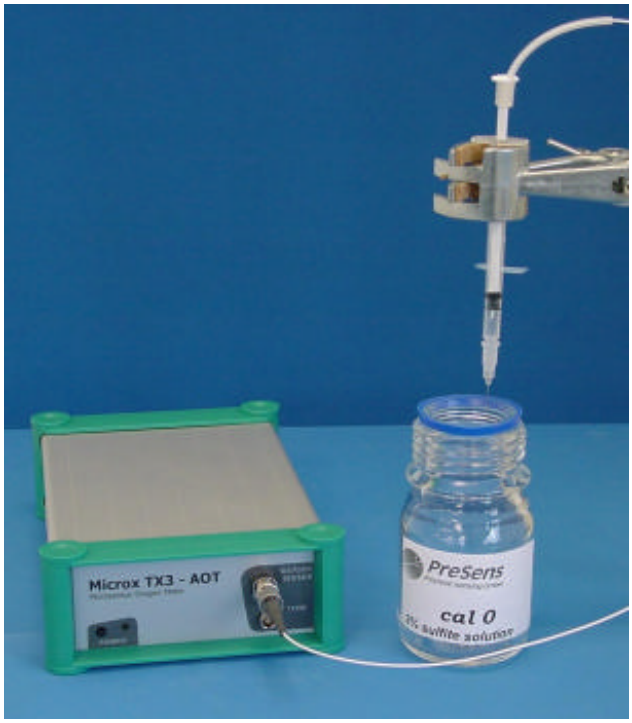


5. Enter the actual **atmospheric pressure** and the **temperature** of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

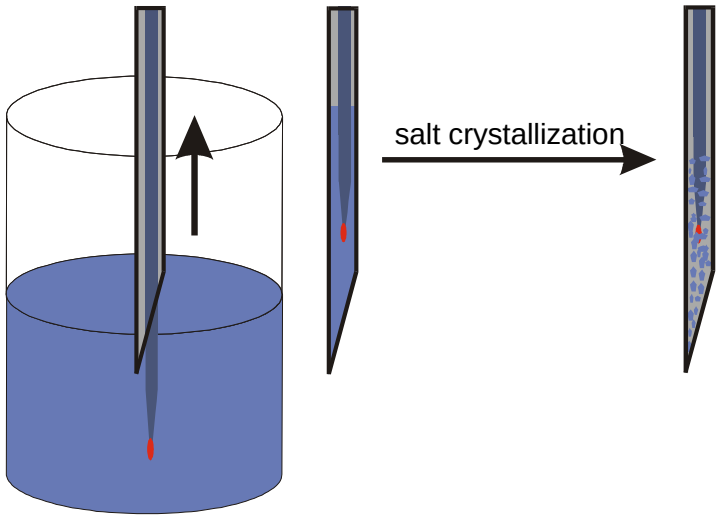


6. Place the vessel with the label '**cal 0**' underneath the microsensor. Please ensure that the glass fiber with its sensor tip is ***not extended***. Locate the syringe needle carefully about 5 mm above the water surface. Slowly press the syringe plunger and extend the glass fiber with its sensor tip from the protective syringe needle.

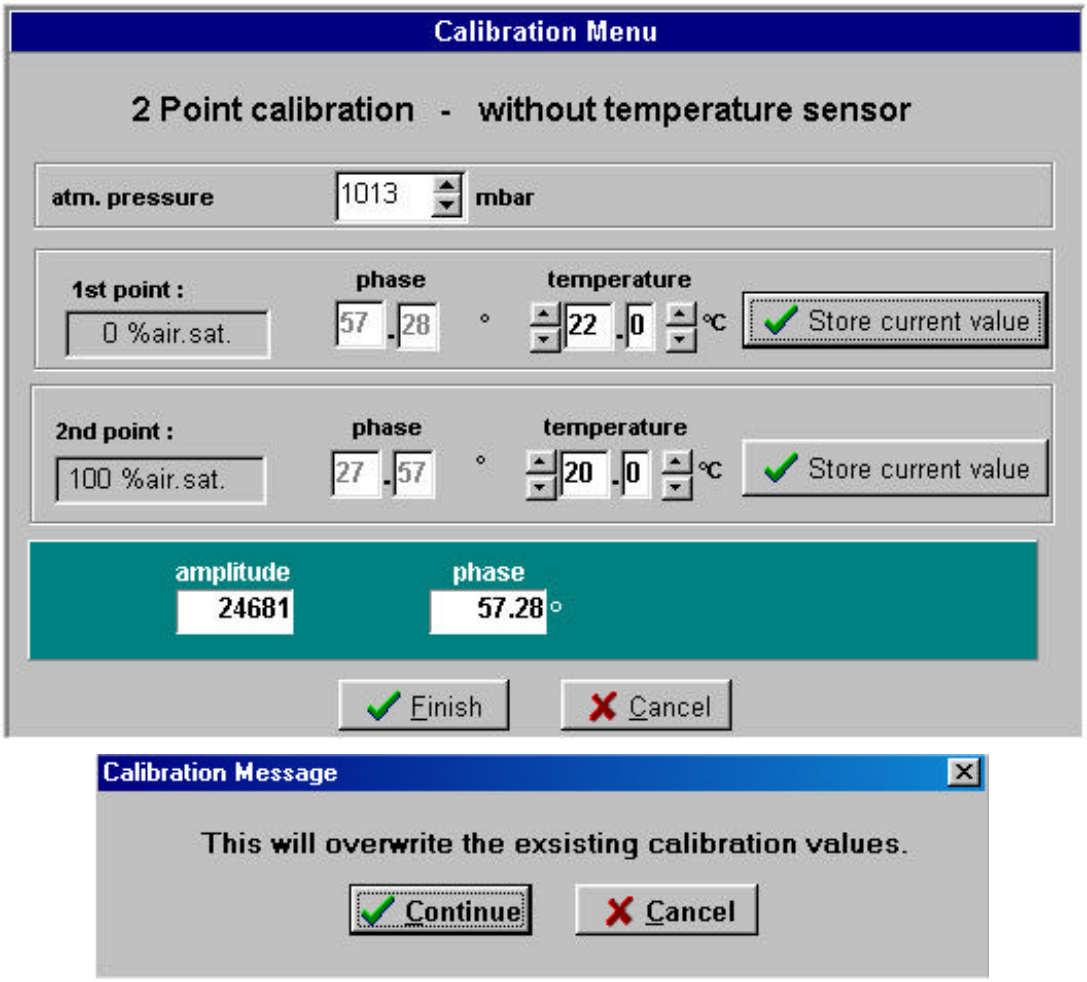
   Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not the protective syringe needle.



If the syringe needle has been dipped into '**cal 0**' by mistake, please wash the glass fiber and the syringe needle with distilled water to avoid salt crystallization within the syringe needle. Salt crystallization may seal the syringe needle and the glass fiber with its sensor tip will break when extended.



7. Wait about 30 sec. until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **0% air-sat.** and **temp. at 0%** values.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



8. Afterwards, wash the glass fiber with its sensor tip with distilled water to clean it from sodium sulfite. Don't retract the sensor tip back into the protective syringe needle. Exchange the calibration solution 0 with an identical vessel filled with distilled water. Make sure not to touch the sensor tip. Dip the sensor tip about 4 mm into the washing solution. Afterwards, retract the glass fiber back into the protective syringe needle without absorbing water.

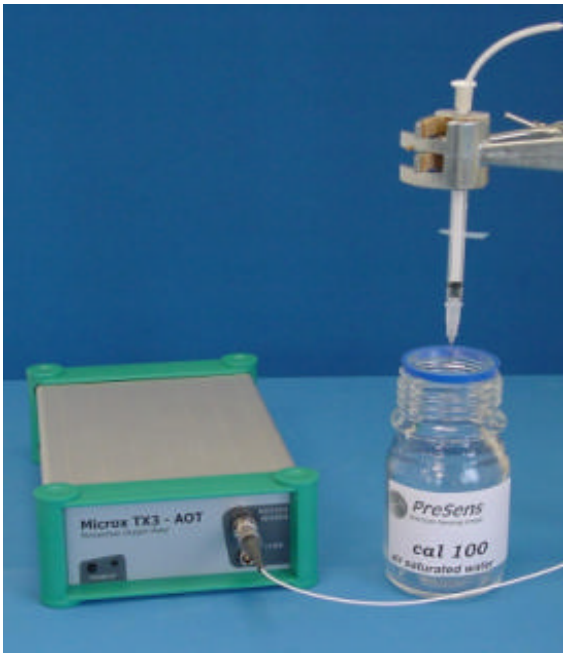
9. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label '**cal 100**' has to be closed by the screw top containing the two holes.

   *Make sure that the glass fiber is **not** extended!*

   Insert the syringe needle through one of the holes until it is about 1 cm deep inside the vessel.

   Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.

   Extend the sensor tip, wait about 30 s until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '***Store current value***' button to store the **100% air-sat.** and **temp. at 100%** values.
   Again, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

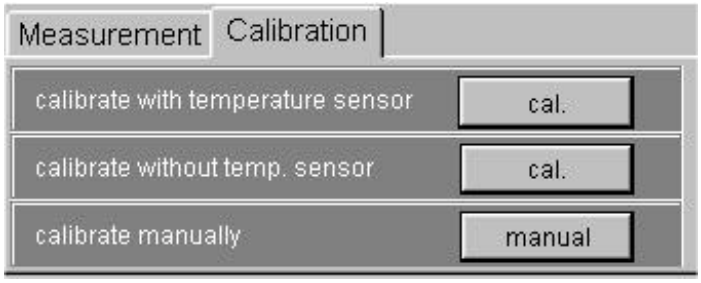


10. Now, calibration is complete. Confirm the calibration values by clicking the '***Finish'*** button.
11. Pull the sensor tip back into its protective syringe needle before removing the microsensor from the calibration vessel.
12. Protect the syringe plunger against slipping out by inserting the transport block back into the syringe housing and don't remove it again until just before measurement.

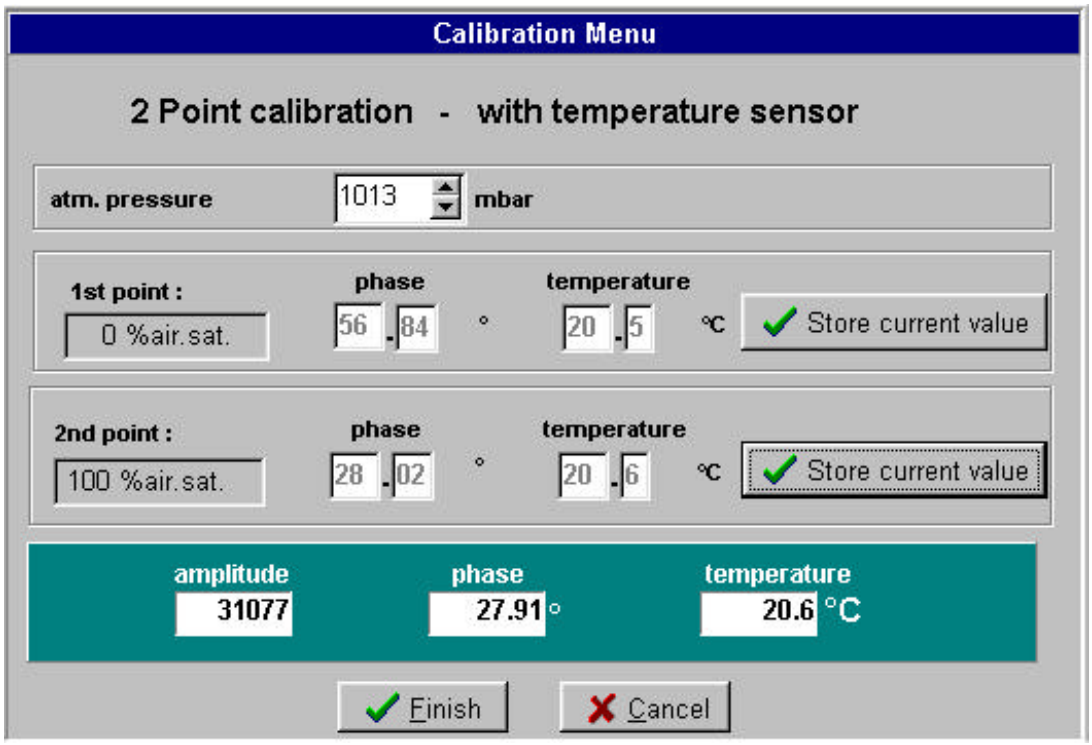
7.1.4 Calibration with Automatic Temperature Compensation

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. To perform temperature compensated measurement, connect the temperature sensor Pt 1000 to the 4-pin connector in front of the Microx TX3. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.

3. Switch on the Microx TX3 and connect the microsensor as shown in Chapter 7.1.2 '***Mounting the Needle-Type Microsensors***'.
4. Start the Microx TX3 software on your computer and click the calibration menu item.
5. Select the calibration routine: '***calibrate with temperature sensor***' by clicking the '***cal.***' button.



6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation in partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.

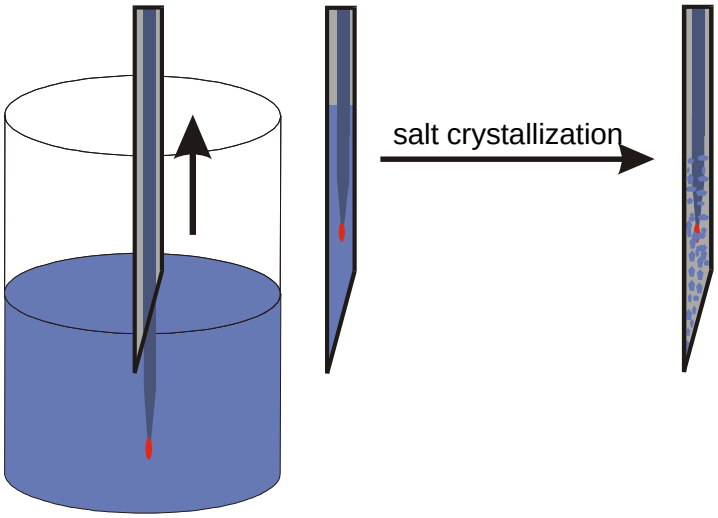


7. Place the vessel with the label '**cal 0**' underneath the microsensor (see picture below). Please ensure that the sensor tip is ***not extended***. Locate the syringe needle carefully about 5 mm above the water surface. Slowly press the syringe plunger and extend the sensor tip from its protective syringe needle.

   Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not its protective syringe needle.

   Ensure that the temperature sensor has been dipped about 1-2 cm into the calibration solution.

If the needle has been dipped into '**cal 0**' by mistake, please wash it with distilled water to avoid salt crystallization within the syringe needle. Salt crystallization may seal the syringe needle and the glass fiber with its sensor tip will break when extended.



8. Wait about 30 sec. until the phase angle and the temperature value is constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the '***Store current value***' button to store both the '***0% air-sat.***' and the temperature '***temp at 0%***'.

   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

9. Afterwards, wash the sensor tip with distilled water to clean it from sodium sulfite. Don't retract the glass fiber back into the protective syringe needle. Exchange the calibration solution 0 with an identical vessel filled with distilled water. Make sure not to touch the glass fiber. Dip the sensor tip about 4 mm into the washing solution. Afterwards, retract the glass fiber back into the protective syringe needle without absorbing water.

   Also wash the temperature sensor by dipping it into water.

10. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label '**cal 100**' has to be closed with the screw top containing the two holes.

    *Make sure that the glass fiber with is sensor tip is **not** extended!*

    Insert the syringe needle through one of the holes until it is about 1 cm deep inside the vessel.

    Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.

    Insert the temperature sensor through the other hole and make sure that it doesn't touch the microsensor. Extend the glass fiber with its sensor tip, wait about 30 s until the phase angle and the temperature is constant (the variation of the phase angle and

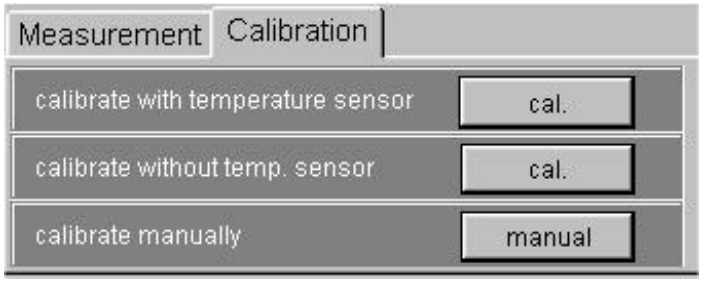
temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '***Store current value***' button to store the '**100% air-sat.**' and **temp. at 100%** values.
A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

11. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Finish***' button.
12. Pull the glass fiber with its sensor tip back into its protective syringe needle before removing the microsensor from the calibration vessel.
13. Protect the syringe plunger against slipping out by inserting the transport block back into the syringe housing.

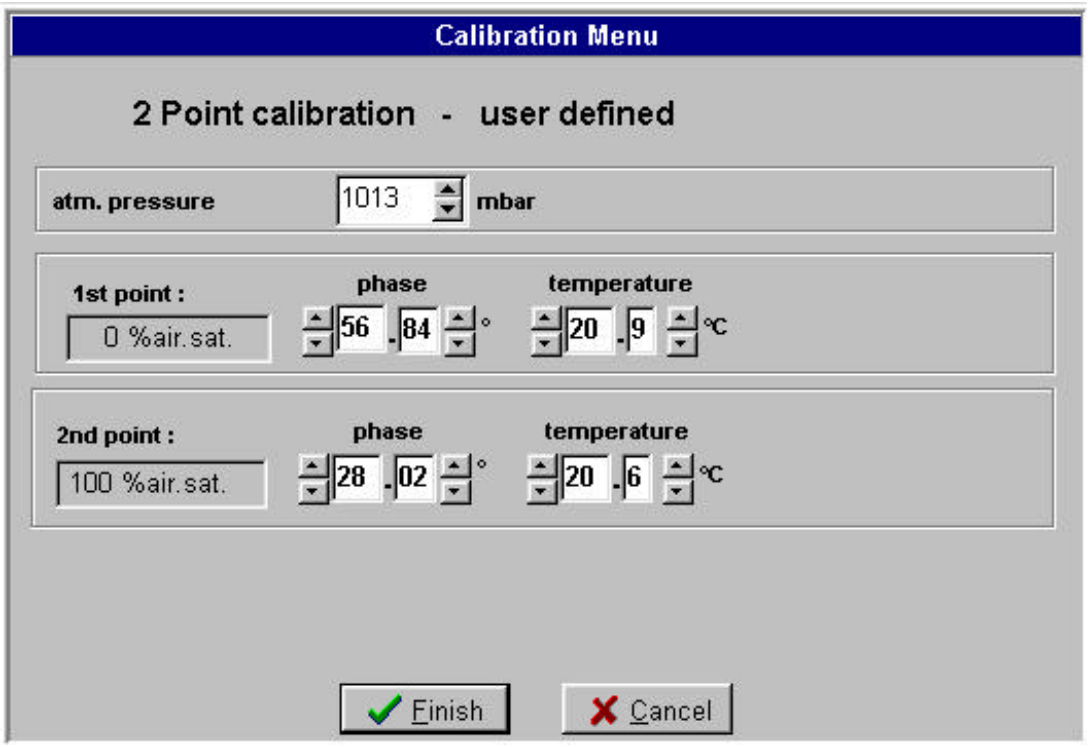
7.1.5 Manual Calibration

A manual calibration should be applied, if you don't want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. Switch on the Microx TX3 oxygen meter
3. Start the Microx TX3 software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' and click the **manual** button



5. Enter the **atmospheric pressure** at which calibration was performed (not the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now, user-defined calibration is complete. Confirm the calibration values by clicking the **Finish** button. A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

7.2 Calibration of Flow-Through Housed Oxygen Microsensors

7.2.1 Preparation of the Calibration Standards

Calibration of microsensors is performed using conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***air-saturated water*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add one gram sodium sulfite(Na_2SO_3) to the vessel and label it '**cal 0**';
2. Dissolve Na_2SO_3 in 100 mL water.
   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is removed by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water is oxygen-free.

Close the vessel after calibration with a screw top to minimize oxygen contamination

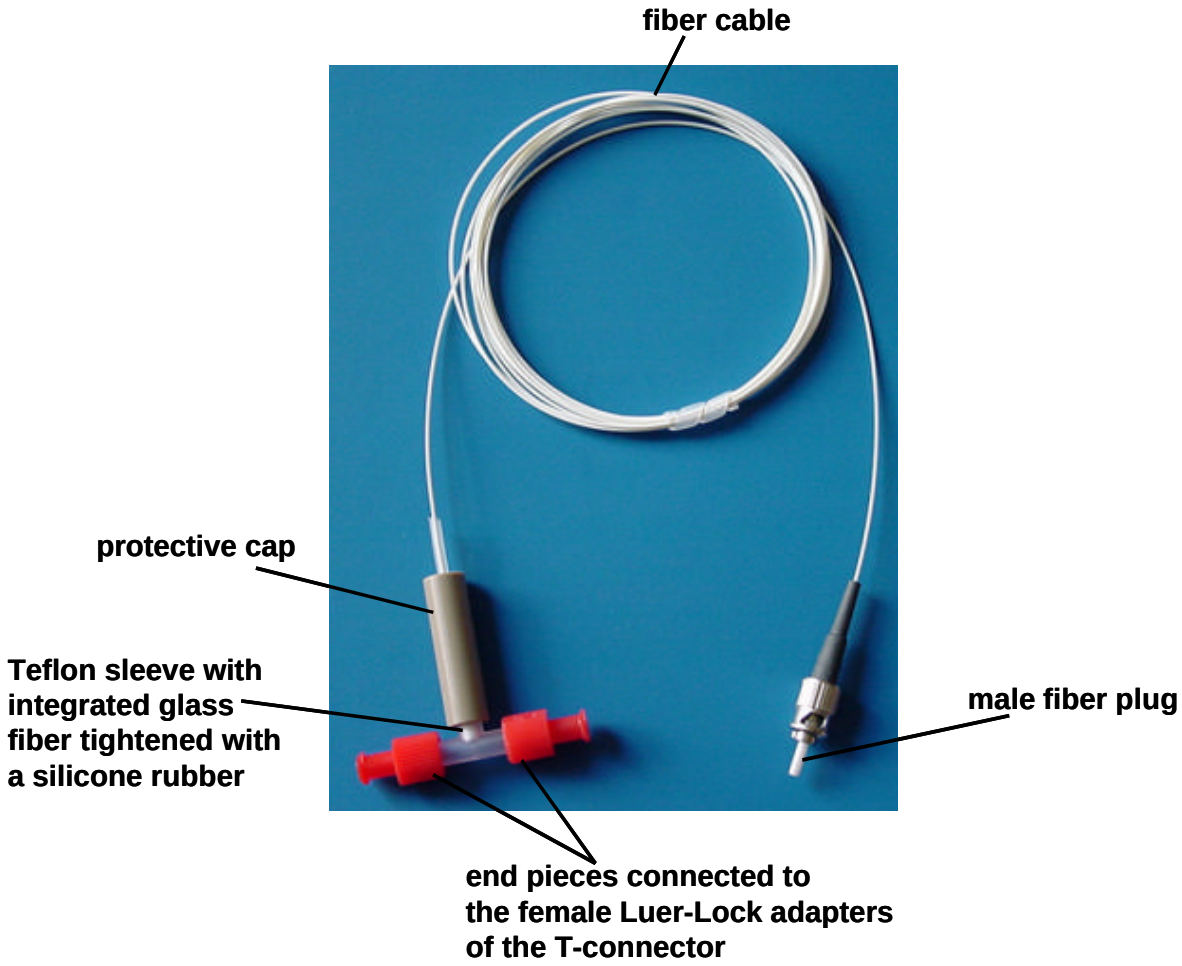
To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of '**cal 0**' is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration solution 100 (air-saturated water)

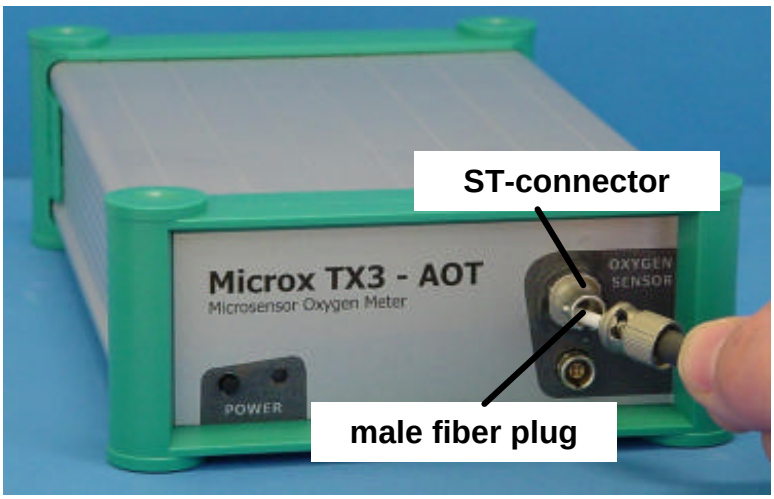
1. Add 100 mL water to a suitable vessel and label it '**cal 100**'.
2. To obtain air-saturated water, blow air into the water using an air-pump with a glass-frit (airstone), creating a multitude of small air bubbles, while stirring the solution.
3. After 20 minutes, switch of the air-pump and stir the solution for further 10 minutes to ensure that water is not supersaturated.

7.2.2 Mounting the Flow-Through Housed Oxygen Microsensors

1. Remove the flow-through housed microsensor carefully from the protective cover. The microsensor is integrated in a sleeve consisting of Teflon tightened with a silicone rubber, which is mounted to a T-connector. The sleeve is protected with a plastic cap screwed to the T-connector. The T-connector has two female Luer-Lock adapters for connection with plastic tubings.


fiber cable
protective cap
Teflon sleeve with integrated glass fiber tightened with a silicone rubber
male fiber plug
end pieces connected to the female Luer-Lock adapters of the T-connector

2. Fix the microsensor with a clip to a laboratory support or a similar stable construction.
3. Remove the two red end-pieces from the T-connector and connect the female Luer-Lock adapters with the tubings of your flow-through system.
4. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the Microx TX3 device. The female fiber-plug of the Microx TX3 has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.

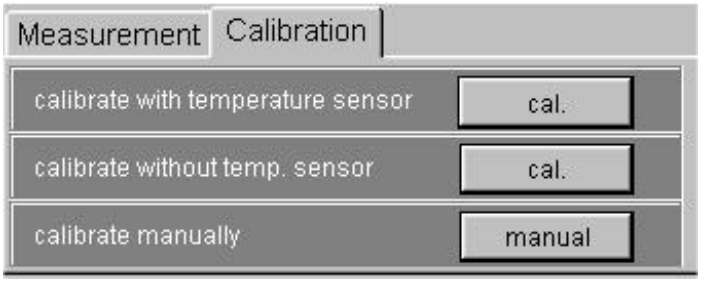


7.2.3 Calibration without Automatic Temperature Compensation

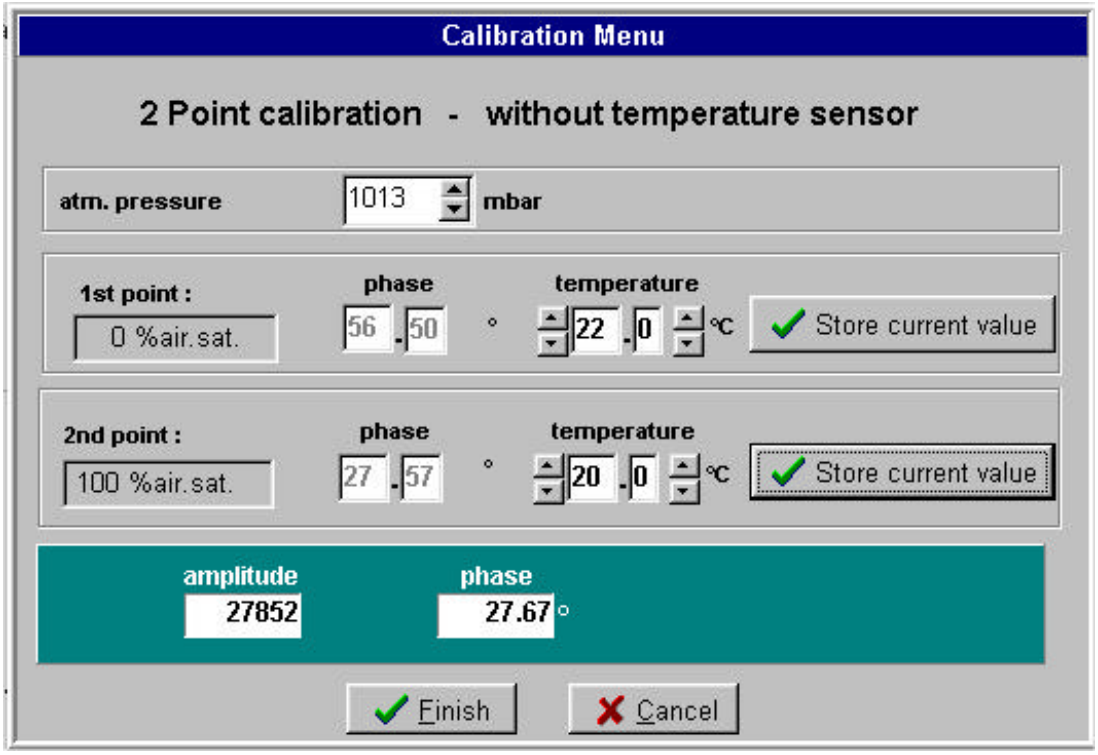
Using the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor PT 1000 is not connected to the Microx TX3.

Please note:
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen microsensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

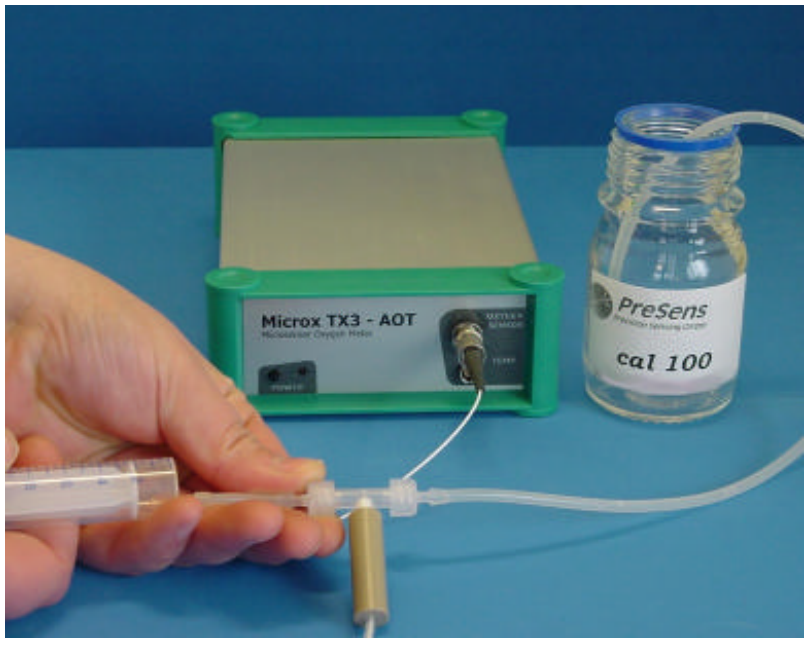
1. Connect the Microx TX3 via the RS232 cable to your computer.
2. Switch on the Microx TX3 and connect the microsensor as shown in Chapter 7.2.2 '***Mounting the Flow-Through Housed Microsensors***'.
3. Start the Microx TX3 software on your computer and click the '***calibration*** ' menu item.
4. Select the calibration routine: '**calibrate without temp. sensor**' and click the '***cal.***' button

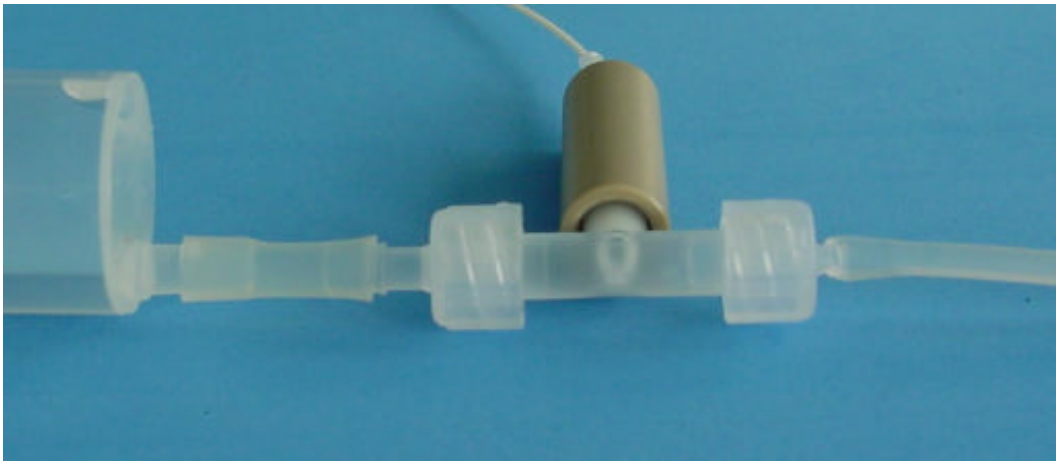


5. Enter the '***actual atmospheric pressure***' and the '***temperature***' of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



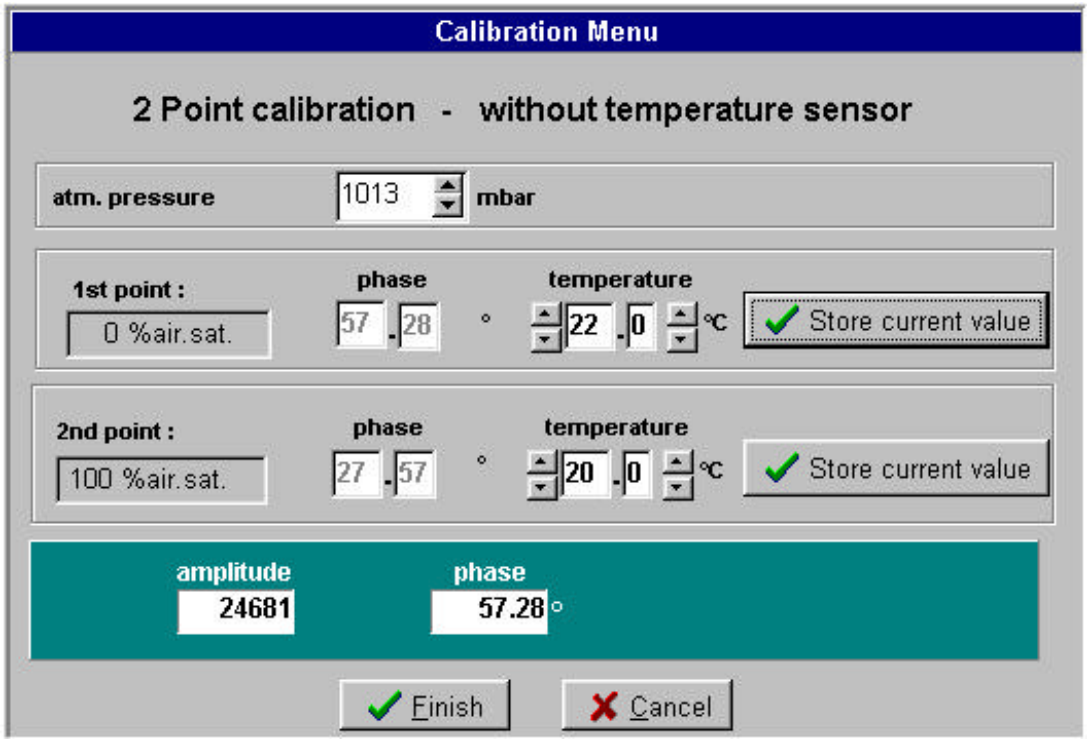
6. Connect one of female Luer-Lock adapters with a plastic tubing which dips into the vessel containing the calibration solution 100, '**cal 100**'. Connect a syringe to the other female Luer-Lock adapter and fill the syringe slowly with calibration solution 100. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.





7. Wait about 30 sec. until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **100% air-sat.** and **temp. at 100%** values. Afterwards, press the calibration solution into the waste.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

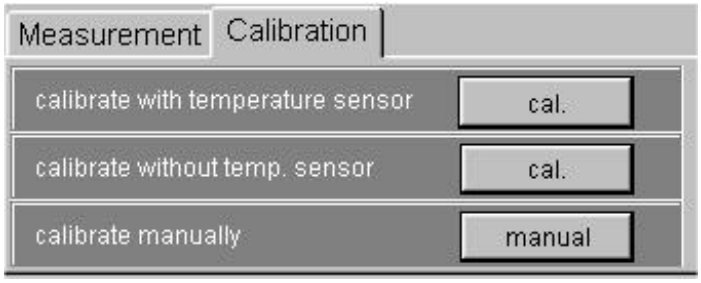
8. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, '**cal 0**' and fill the syringe slowly with it. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.
   Wait about 30 s until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '**Store *current value***' button to store the **0% air-sat.** and **temp. at 0%** values. Afterward, press the calibration solution into the waste.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



9. Now, calibration is complete. Confirm the calibration values by clicking the '***Finish'*** button.
10. Now you have to wash the glass fiber with its sensor tip with distilled water to clean it from sodium sulfite. Dip the plastic tubing into a vessel containing distilled water and fill the syringe. Press the washing solution to the waste, not back into the vessel. Repeat this washing procedure 3 times.

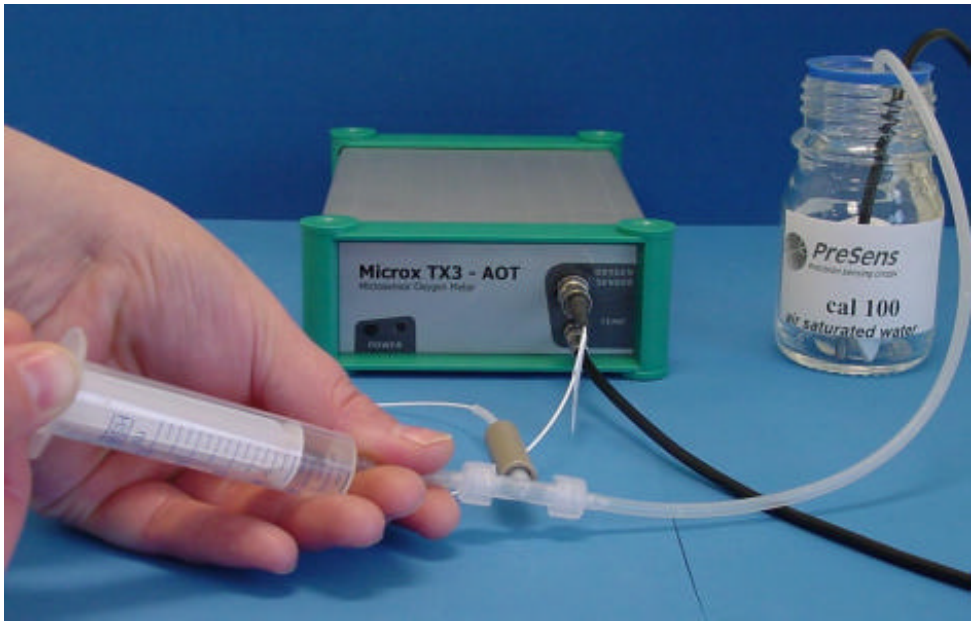
7.2.4 Calibration with Automatic Temperature Compensation

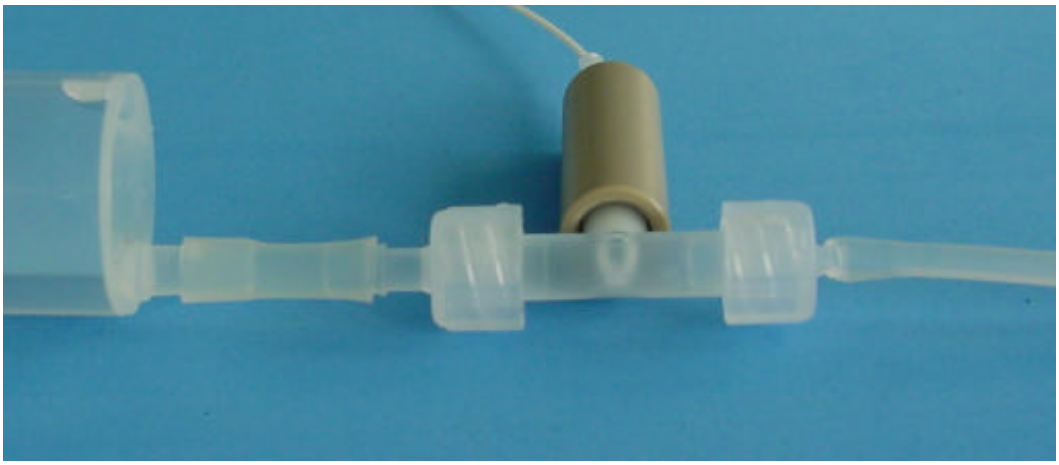
1. Connect the Microx TX3 via the RS232 cable to your computer.
2. To perform temperature compensated measurement, connect the temperature sensor PT 1000 to the 4-pin connector in front of the Microx TX3.
3. Switch on the Microx TX3 and connect the microsensor as shown in Chapter 7.2.2 '***Mounting the Flow-Through Housed Microsensors***'.
4. Start the Microx TX3 software on your computer and click the calibration menu item.
5. Select the calibration routine: '***calibrate with temperature sensor***' and click the '***cal.***' button.



6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.
7. Connect one of female Luer-Lock adapters with a plastic tubing which dips into the vessel containing the calibration solution 100, '**cal 100**'. Connect a syringe to the other female Luer-Lock adapter and fill the syringe slowly with calibration solution 100. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.

   Ensure that the temperature sensor has been dipped into the calibration solution '**cal 100**' and that there are no temperature differences between the calibration vessel and the flow-through cell.

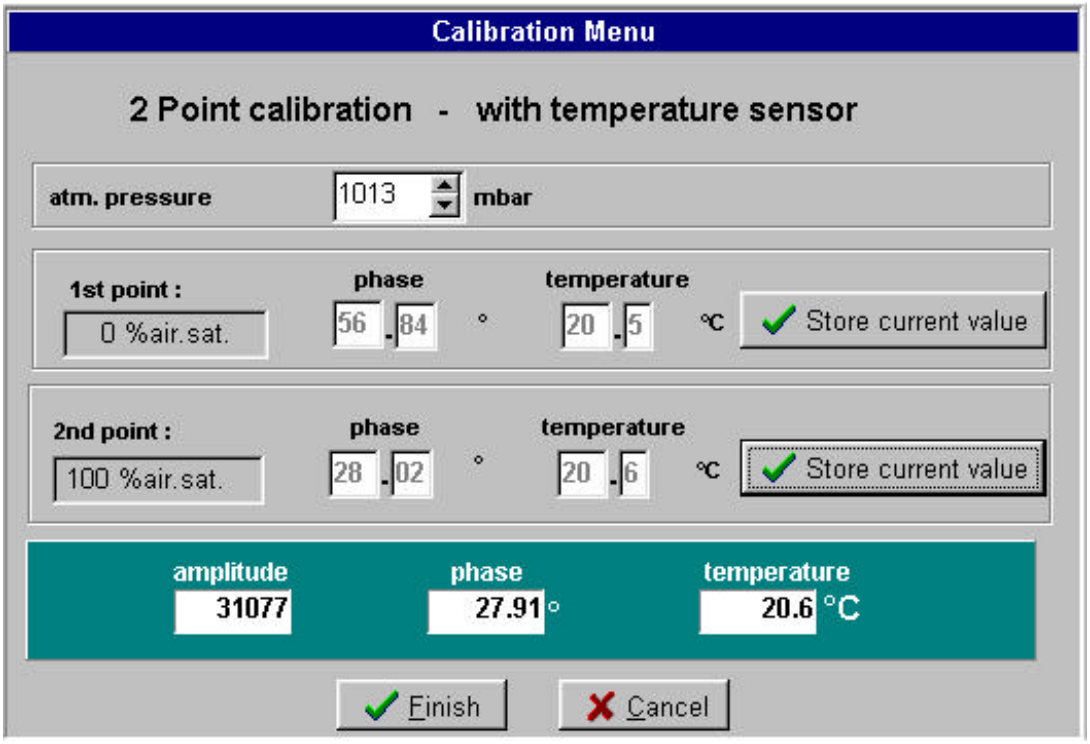




8. Wait about 30 sec. until the phase angle and the temperature value is constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the '***Store current value***' button to store both the ***100% air-sat.*** and its temperature '***temp at 100%***'. Afterwards, press the calibration solution back the waste.
   A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

9. To record the second calibration value, oxygen-free water, dip the plastic tubing into the vessel containing the calibration solution 0, '**cal 0**' and fill the syringe slowly with it. Please ensure that there are no air-bubbles located in the T-connector around glass fiber with its sensitive tip.
   Ensure that the temperature sensor has been dipped into the calibration solution '**cal 0**'.

10. Wait about 30 s until the phase angle and the temperature is constant (the variation of the phase angle and temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '***Store current value***' button to store the **0% air-sat.** and **temp. at 0%** values. Afterwards, press the calibration solution into the waste.
    Again, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

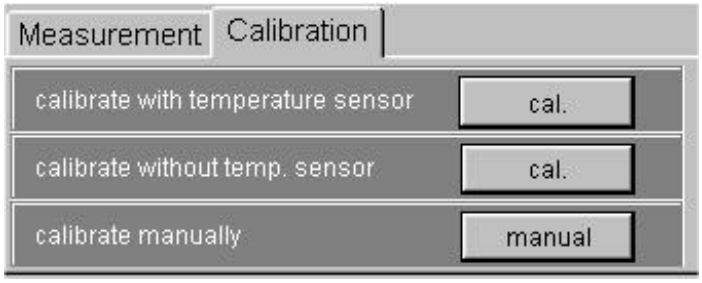


11. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Finish'*** button.
12. Now you have to wash the glass fiber with its sensor tip with distilled water to clean it from sodium sulfite. Dip the plastic tubing into a vessel containing distilled water and fill the syringe. Press the washing solution to the waste, not back into the vessel. Please repeat this washing procedure 3 times.
    Also wash the temperature sensor by dipping it into water.

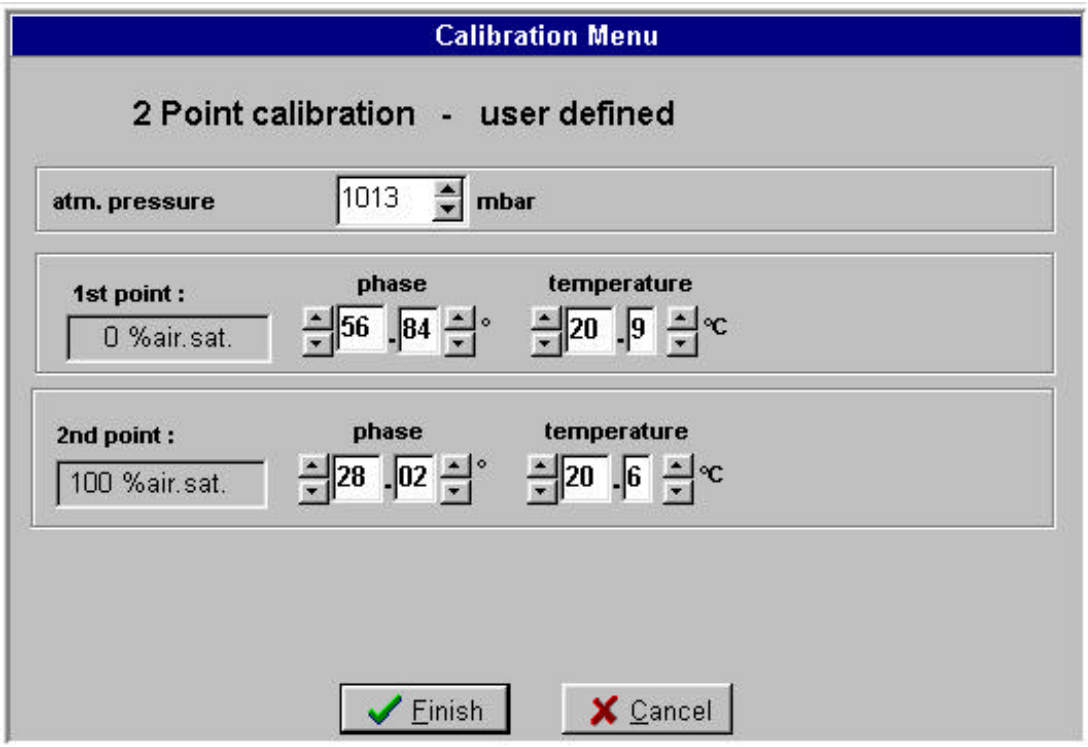
7.2.5 Manual Calibration

A manual calibration should be applied, if you don't want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. Switch on the Microx TX3 oxygen meter
3. Start the Microx TX3 software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' and click the **manual** button



5. Enter the **atmospheric pressure** at which calibration was performed (not the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now, user-defined calibration is complete. Confirm the calibration values by clicking the '**Finish**' button. When doing so, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

7.3 Calibration of Implantable Oxygen Microsensors

7.3.1 Preparation of the Calibration Standards

Calibration of microsensors is performed using conventional two-point calibration in ***oxygen-free water*** (cal 0) and ***water-vapor saturated air*** (cal 100).

Preparation of calibration solution 0 (oxygen-free water):

1. Add one gram sodium sulfite(Na_2SO_3) to the vessel and label it '**cal 0**';
2. Dissolve Na_2SO_3 in 100 mL water.
   Water becomes oxygen-free due to a chemical reaction of oxygen with Na_2SO_3. Additional oxygen, diffusing from air into the water, is eliminated (removed) by surplus of Na_2SO_3.
3. Close the vessel with a screw top and shake it for approximately one minute to dissolve Na_2SO_3 and to ensure that water is oxygen-free.

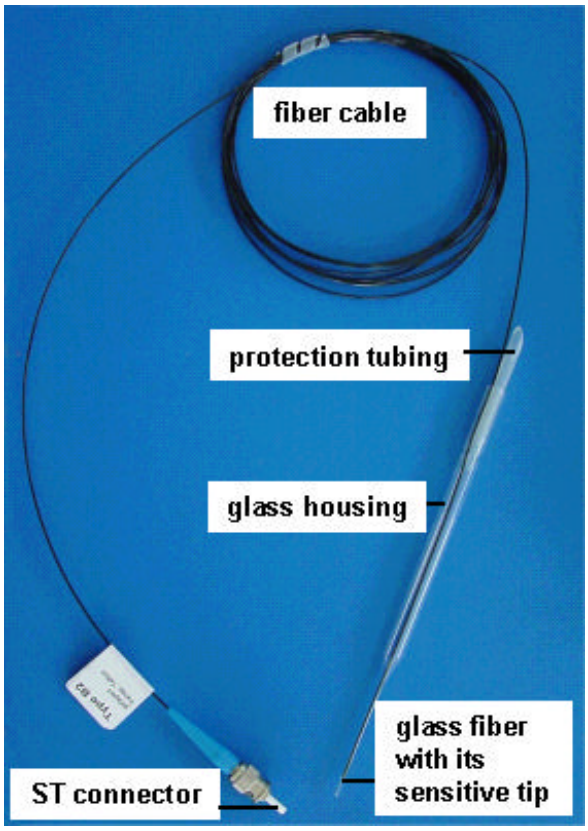
Close the vessel after calibration with a screw top to minimize oxygen contamination
To prepare oxygen-free water you also can use sodium dithionit ($Na_2S_2O_4$). The shelf life of '**cal 0**' is about 24 hours provided that the vessel has been closed with the screw top.

Preparation of calibration standard 100 (water-vapor saturated air)

1. Place wet cotton wool in the vessel and label it '**cal 100**'.
2. Drill two holes for inserting the microsensor and the temperature sensor in the screw top and close the vessel with it.
3. Wait about 2 minutes to ensure that air is water-vapor saturated

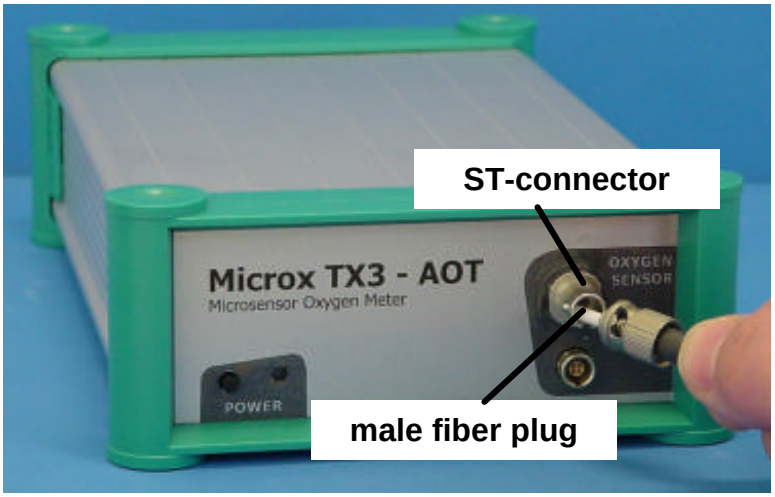
7.3.2 Mounting the Implantable Microsensors

1. Remove the microsensor carefully from the protective cover. The microsensor is protected with a glass housing during the transport.
2. Fix the glass housing microsensor with a clip to a laboratory support or a similar stable construction.



We strongly advise you not to handle with microsensors without the support - especially when the sensor tip is extended.

3. Remove the protective cap from the male fiber plug and connect it to the ST-plug of the Microx TX3 device. The female fiber-plug of the Microx TX3 has a groove in which the spring of the male fiber-plug of the microsensor has to be inserted. The safety nut must be carefully attached while turning and is locked by turning slightly clockwise. Be careful not to snap off the fiber cable.

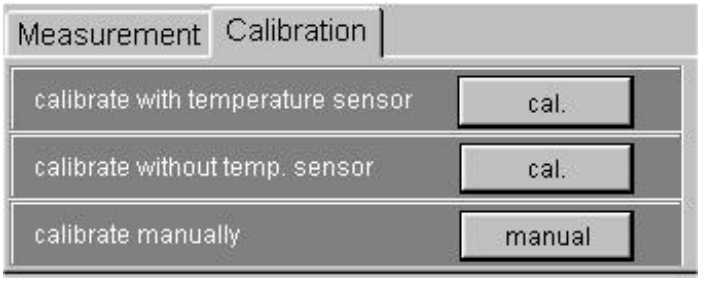


7.3.3 Calibration without Automatic Temperature Compensation

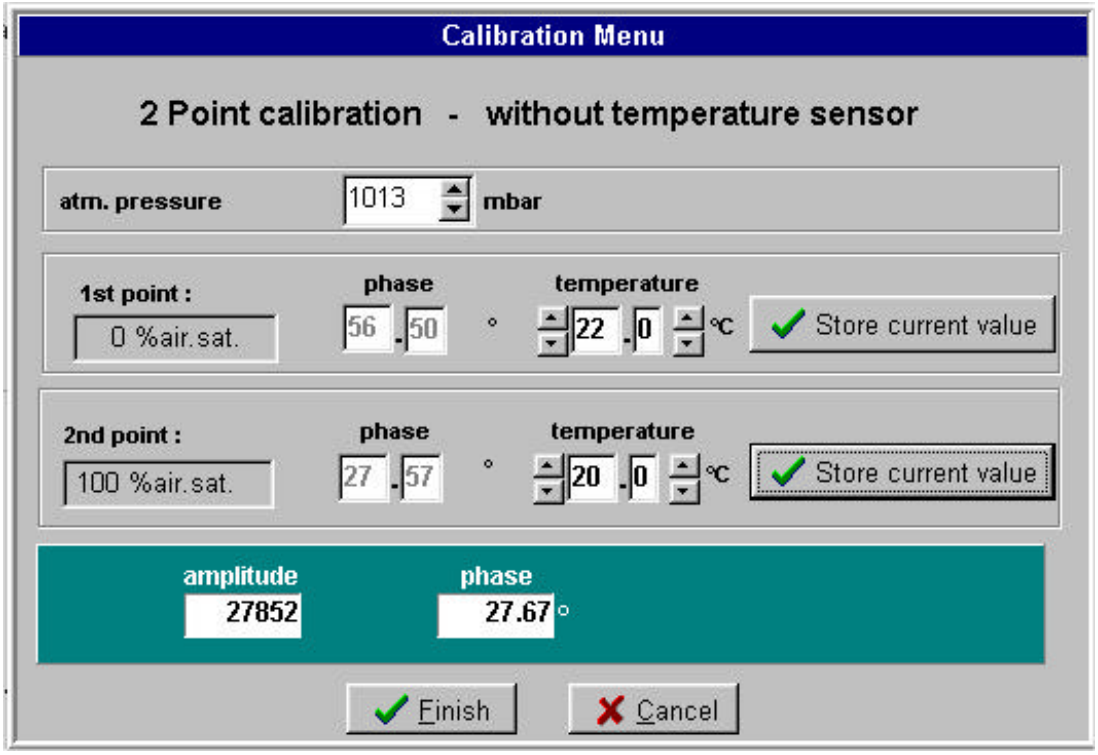
Using the software, you can choose whether to perform the measurement and calibration with or without temperature compensation. If you want to perform the calibration without automatic temperature compensation, please ensure that the delivered temperature sensor PT 1000 is not connected to the Microx TX3.

Please note:
Calibration without temperature compensation only makes sense if there is no temperature change during the calibration of the oxygen microsensor. Besides, it must be ensured that the temperature during later measurement is constant and already known. However, the temperatures during the measurement and the calibration process are allowed to be different.

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. Switch on the Microx TX3 and connect the microsensor as shown in Chapter 7.3.2 '***Mounting the Implantable Microsensors***'.
3. Start the Microx TX3 software on your computer and click the '***calibration***' menu item.
4. Select the calibration routine: '***calibrate without temp. sensor***' and click the '***cal.***' button

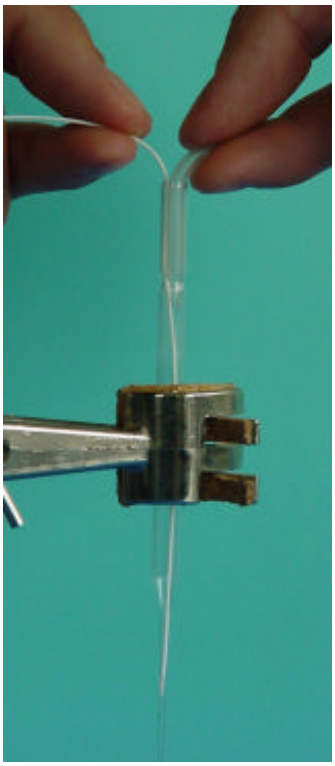


5. Enter the actual '***atmospheric pressure***' and the '***temperature***' of the calibration standards. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.



6. Place the vessel with the label '**cal 0**' underneath the microsensor. Please ensure that the glass fiber with its sensor tip is in the protective glass housing. Locate the glass housing carefully about 5 mm above the water surface.

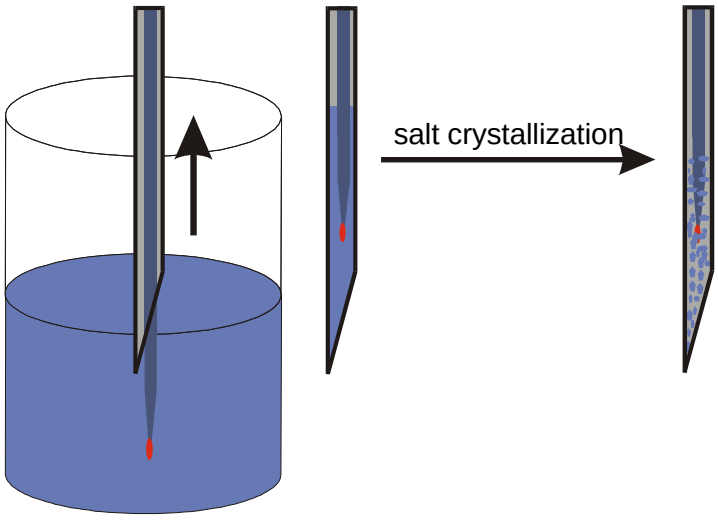
7. The glass fiber with its sensing tip is prevented from slipping using a protection tubing. Slacken the protection tubing from the glass housing, extend the sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing



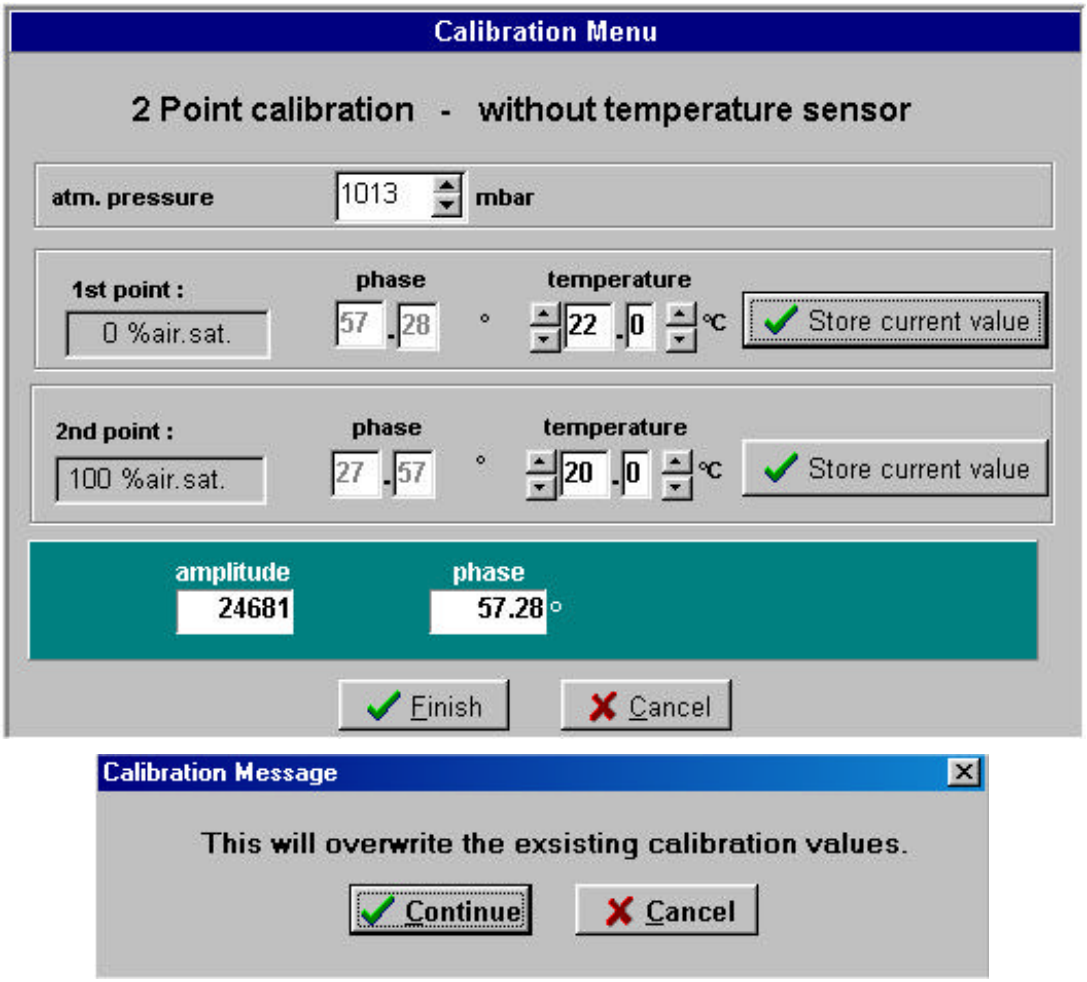
WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK!

8. Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not the protective glass housing.

If the glass housing has been dipped into '**cal 0**' by mistake, please wash the glass fiber and the glass housing with distilled water to avoid salt crystallization within the housing. Salt crystallization may seal the housing and the glass fiber with its sensor tip will break when extended.



9. Wait about 30 sec. until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and press the '***Store current value***' button to store the **0% air-sat.** and **temp. at 0%** values.
A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



10. Afterwards, wash the sensor tip with distilled water to clean it from sodium sulfite. Make sure not to touch the sensor tip. Retract the glass fiber back into the protective glass housing without absorbing water.

11. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The vessel with the label '**cal 100**' has to be closed with the screw top containing the two holes.

    *Make sure that the glass fiber is **not** extended!*

    Insert the glass housing through one of the holes until it is about 1 cm deep inside the vessel.

    Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.

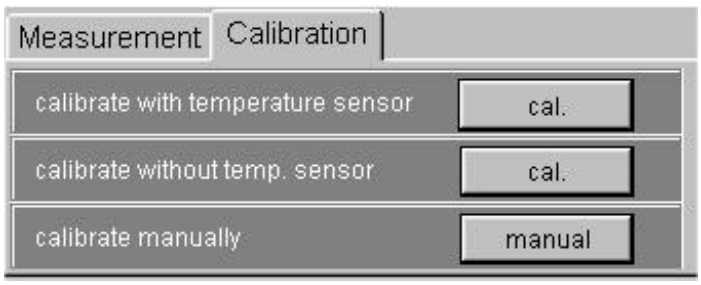
    Slacken the protection tubing from the glass housing, extend the sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing.

    Wait about 30 s until the phase angle is constant (the variation of the phase angle should be smaller than ± 0.05°) and click the '***Store current value***' button to store the **100% air-sat.** and **temp. at 100%** values.
    A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

12. Now, calibration is complete. Confirm the calibration values by clicking the '***Finish***' button.
13. Pull the glass fiber with its sensor tip back into its protective glass housing before removing the microsensor from the calibration vessel.

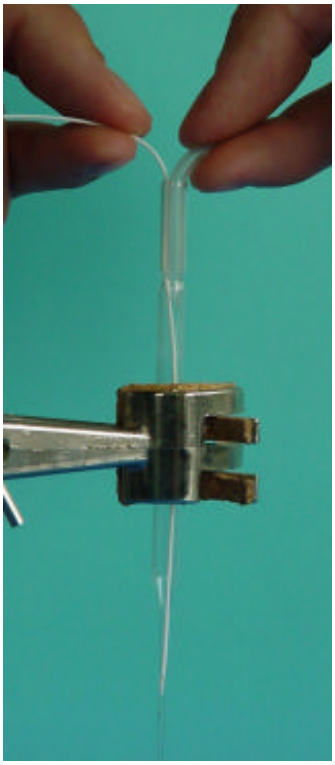
7.3.4 Calibration with Automatic Temperature Compensation

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. To perform temperature compensated measurement, connect the temperature sensor Pt 100 to the 4-pin connector in front of the Microx TX3. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.
3. Switch on the Microx TX3 and connect the microsensor as shown in Chapter 7.3.2 '***Mounting the Microsensors***'.
4. Start the Microx TX3 software on your computer and click the calibration menu item.
5. Select the calibration routine: '***calibrate with temperature sensor***' and click the '**cal.**' button.



6. Enter the '***actual atmospheric pressure***'. The atmospheric pressure of the calibration is needed to convert the oxygen unit % air-saturation into partial pressure units (hPa, Torr) or concentrations units (mg/L µmol/L). Please note, that changes in the actual atmospheric pressure have no effect on the partial pressure units (hPa, Torr) and concentrations units (mg/L, µmol/L) but the oxygen units % air-saturation and % oxygen-saturation have to be corrected for air pressure changes.
7. Place the vessel with the label '**cal 0**' underneath the microsensor. Please ensure that the sensor tip is in the protective glass housing. Locate the glass housing carefully about 5 mm above the water surface.

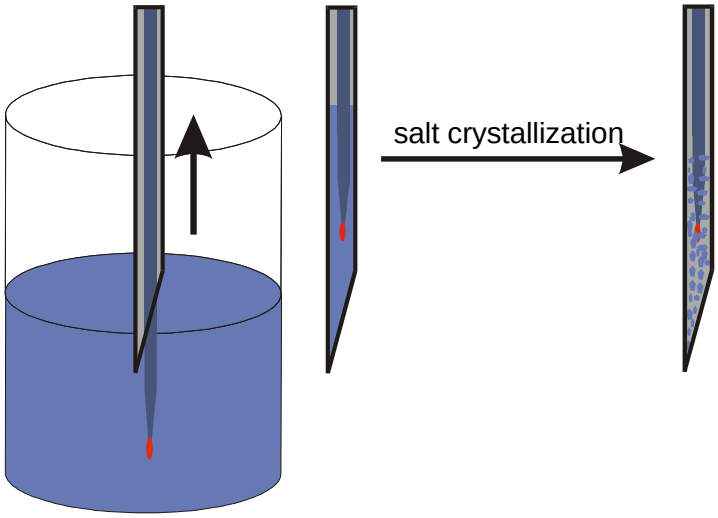
   Ensure that the temperature sensor has been dipped about 1-2 cm into the calibration solution.
8. The sensing tip is prevented from slipping using a protection tubing. Slacken the protection tubing from the glass housing, extend the sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing.



WHEN GLASS-FIBER WITH ITS SENSOR TIP IS PUSHED OUT, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK!

9. Ensure that the sensor tip is dipped about 4 mm into the calibration solution 0, but not the protective glass housing.

If the glass housing has been dipped into '**cal 0**' by mistake, please wash the glass fiber and the glass housing with distilled water to avoid salt crystallization within the housing. Salt crystallization may seal the housing and the glass fiber with its sensor tip will break when extended.



10. Wait about 30 sec. until the phase angle and the temperature value is constant (the variation of the phase angle and the temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and press the '**Store *current value***' button to store both the ***0% air-sat.*** and its temperature ***temp at 0%***.
    A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.
11. Afterwards, wash the sensor tip with distilled water to clean it from sodium sulfite. Make sure not to touch the sensor tip. Retract the glass fiber back into the protective glass housing without absorbing water.

    Also wash the temperature sensor by dipping it into water.
12. Now you have to record the second calibration value, water-vapor saturated air. Place the calibration standard 100, containing wet cotton wool, below the microsensor. The

vessel with the label '**cal 100**' has to be closed with the screw top containing the two holes.

*Make sure that the glass fiber is **not** extended!*

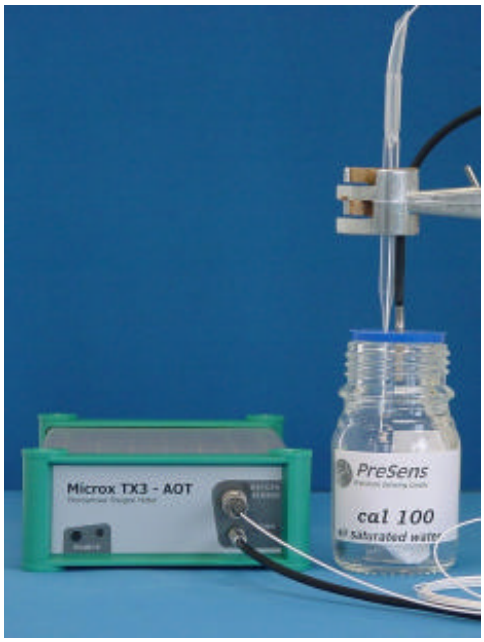
Insert the glass housing through one of the holes until it is about 1 cm deep inside the vessel.

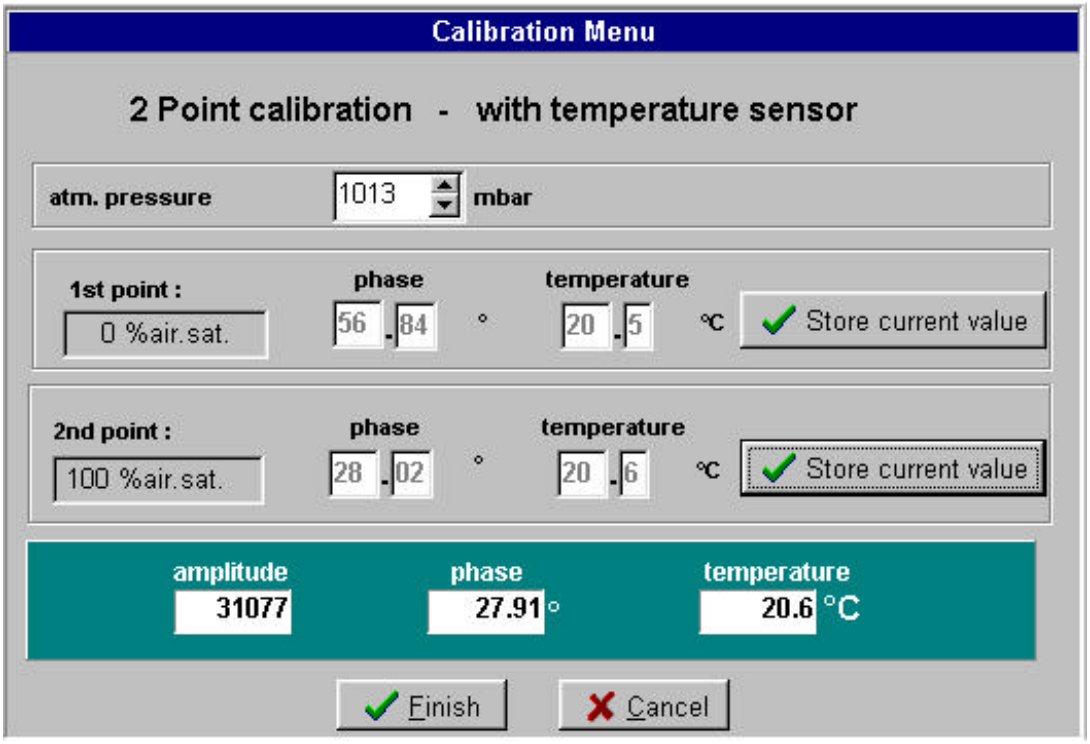
Insert the temperature sensor through the other hole and make sure that it doesn't touch the microsensor.

Make sure that the glass fiber with its sensor tip does not touch the cotton wool when extended.

Slacken the protection tubing from the glass housing, extend the glass fiber with its sensor tip about 1 cm from the glass housing and fix the glass fiber again with the protection tubing.

Wait about 30 s until the phase angle and the temperature is constant (the variation of the phase angle and temperature should be smaller than ± 0.05° and 0.2 °C, respectively) and click the '**Store *current value***' button to store the **100% air-sat.** and **temp. at 100%** values.
A message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.



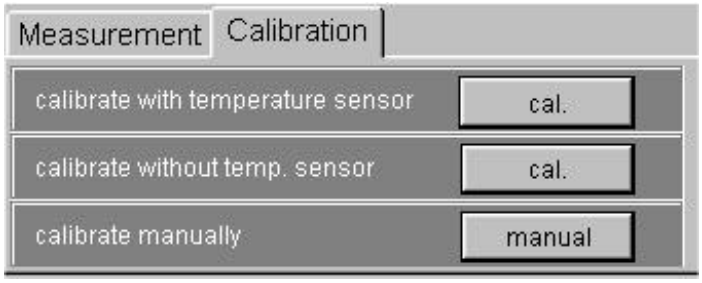


13. Now, calibration with temperature compensation is complete. Confirm the calibration values by clicking the '***Store'*** button.
14. Pull the glass fiber with its sensor tip back into its protective glass housing before removing the microsensor from the calibration vessel.

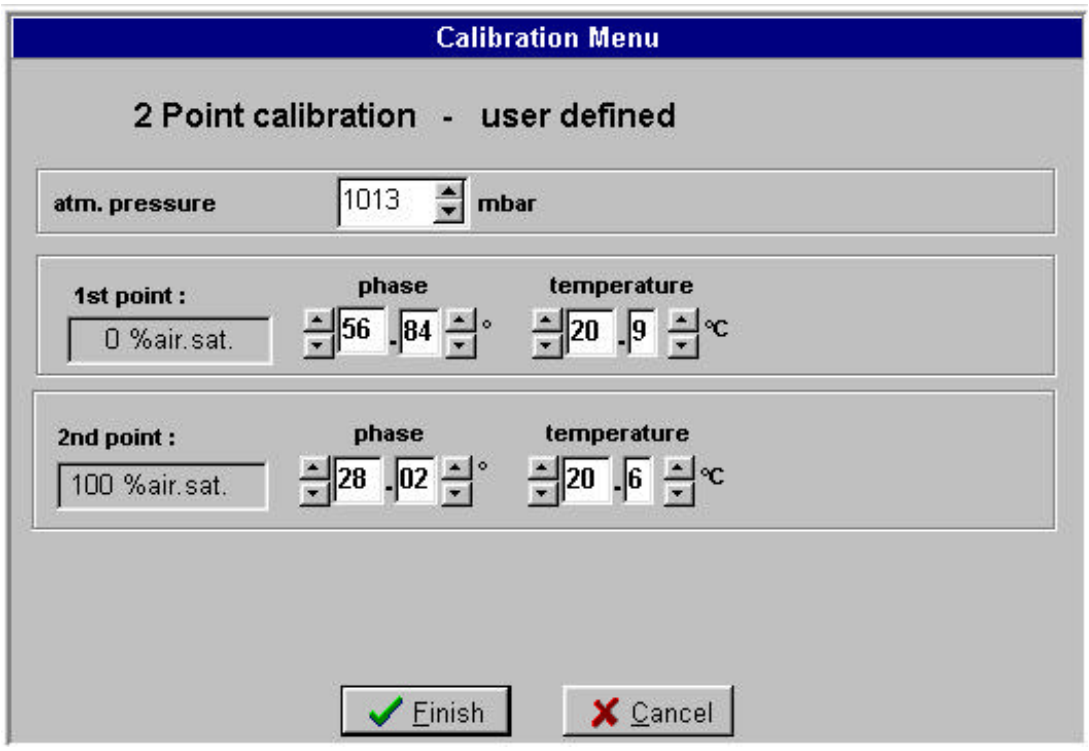
7.3.5 Manual Calibration

A manual calibration should be applied, if you don't want to calibrate your sensor again. However, this is only possible if you already know the calibration values of the special sensor.

1. Connect the Microx TX3 via the RS232 cable to your computer.
2. Switch on the Microx TX3 oxygen meter
3. Start the Microx TX3 software on your computer and click the **Calibration** menu item.
4. Select the calibration routine '**calibrate manually**' and click the **manual** button



5. Enter the **atmospheric pressure** at which calibration was performed (not the **actual** one) and the respective calibration values **0% air-sat., temp. at 0%** and **100 % air-sat., temp. at 100%**.



6. Now, user-defined calibration is complete. Confirm the calibration values by clicking the '**Finish**' button. When doing so, a message window opens and informs you that you will overwrite the existing calibration values. Click the '***Continue***' button to store the new calibration data.

8 Measurement

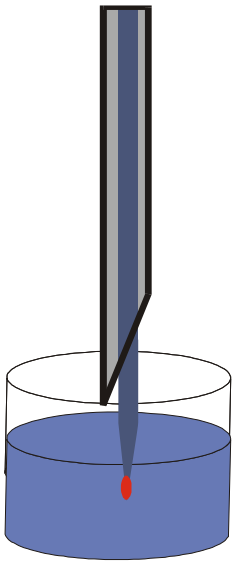
Calibration of the microsensor is recommended before each measurement (see chapter 7 '***Calibration of Microsensors***'). If you don't want to recalibrate the microsensor, you can use the calibration values of your last measurement (see '**User-Defined Calibration**'). Each calibration is only valid for the corresponding microsensor and should be repeated before beginning a new measurement. Especially, after longer measurements (more than 18000 measuring points or 5 h continuous sensor-illumination) the sensor should be re-calibrated.
Ensure that the temperature of the sample is known and is constant during measurement, if you do not use temperature compensation. In the case of temperature-compensated measurements, the temperature sensor Pt 1000 should be positioned as close as possible to the microsensor to avoid temperature differences.

8.1 Measurement with Needle-Type Oxygen Microsensors

1. Please carefully read chapter 7.1.2 '***Mounting the Needle-Type Microsensors***' and chapters 7.1.3, 7.1.4 '***Calibration of the Microsensor without / with Automatic Temperature Compensation***' in the manual. There you will find relevant information about the proper handling of microsensors. They are the basic for the following chapter.
2. Connect the Microx TX3 via the RS232 cable to your computer.
3. Connect the temperature sensor Pt 1000 to the 4-pin connector on the front panel of the Microx TX3, to perform temperature-compensated measurement. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.
4. Calibrate the sensor according to chapter 7.1 '***Calibration of Needle-Type Oxygen Microsensors***'. If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose '***User-Defined***' calibration which is described in chapter 7.1.5.
5. Position the microsensor right above your sample. The syringe needle - ***with retracted glass fiber*** – can be punched through a sept or immersed into a tissue. Remove the transport block .

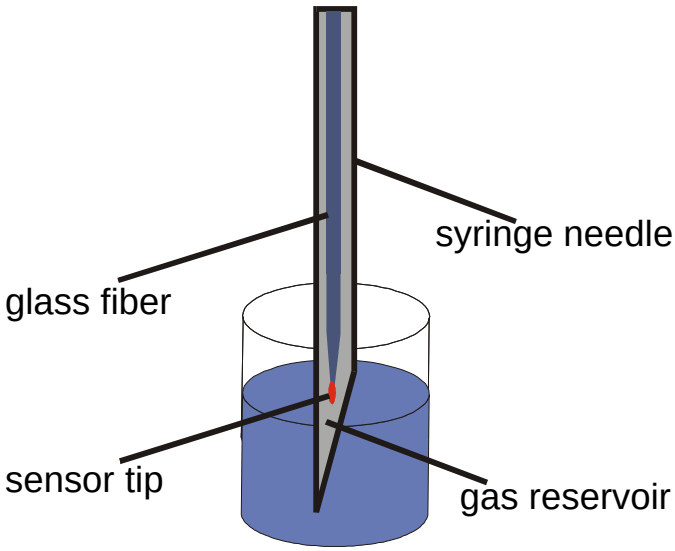
   Extend the glass fiber with its sensor tip from the syringe needle by carefully pressing the syringe plunger. Please take into account that the fine glass fiber with its sensor tip is mechanically quite sensitive! Avoid mechanical stress as far as possible.

6. The sensor tip will only measure accurately if the glass fiber with its sensor tip has been completely extended from the syringe needle. Inside the needle there is an air-reservoir, in which the oxygen content is different to your sample!



right position of the sensor tip!

Sensor tip ***must*** be outside for measurement and calibration!



wrong position of the sensor tip!

There is an air reservoir inside the syringe needle. The oxygen content inside the syringe needle is different to that of the sample, since the gas exchange rate is slow.

7. Please rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Retract the sensor tip into the protective housing and insert the transport block to prevent the syringe plunger from slipping.

8.2 Measurement with Flow-Through Housed Oxygen Microsensors

1. Please carefully read chapter 7.2 '***Calibration of Flow-Through Housed Oxygen Microsensors***' in the manual instructions. There you will find relevant information about the proper handling of microsensors. They are the basic for the following chapter.
2. Connect the Microx TX3 via the RS232 cable to your computer.
3. Connect the temperature sensor Pt 100 to the 4-pin connector on the front panel of the Microx TX3 and carefully tighten the safety nut, to perform temperature-compensated measurement. Immerse the temperature sensor in your sample and fix it with a laboratory support.
4. Calibrate the sensor according to chapter 7.2 '***Calibration of Flow-Through Housed Oxygen Microsensors***'. If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose '***User-Defined***' calibration which is described in chapter 7.2.5.
5. Connect the end-pieces of the T-connector with Luer-Lock tubings (obtained by novodirect B13323, B13312, B13316 B13303) or Luer-Lock adapters for tubings (obtained by novodirect B95900, B95908, B95919) and pump your sample through the flow-through cell.
6. Please rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues.Pump distilled water through the cell until all sample residues are removed.

8.3 Measurement with Implantable Oxygen Microsensors

1. Please carefully read chapter 7.3 '***Calibration of Implantable Oxygen Microsensors***' in the manual instructions. There you will find relevant information about the proper handling of microsensors. They are the basic for the following chapter.
2. Connect the Microx TX3 via the RS232 cable to your computer.
3. Connect the temperature sensor Pt 100 to the 4-pin connector on the front panel of the Microx TX3 and carefully tighten the safety nut, to perform temperature-compensated measurement. Fix the temperature sensor and make sure that neither the temperature sensor nor its cable can touch the microsensor.
4. Remove the microsensor carefully from the protective cover. The microsensor is protected with a glass housing during the transport. Fix the glass housing microsensor with a clip to a laboratory support or a similar stable construction.
5. Calibrate the sensor according to chapter 7.3 '***Calibration of Implantable Oxygen Microsensors***'. If you do not want to re-calibrate the sensor but use the calibration values of your last measurement, choose '***User-Defined***' calibration which is described in chapter 7.3.5.
6. The glass fiber with its sensing tip is prevented from slipping from the glass housing using a protection tubing. Remove the fiber cable from the glass housing for implantation. Slacken the protection tubing from the glass housing and carefully extract the glass. Be careful, don't touch the glass housing with the glass fiber tip.

WHEN GLASS-FIBER WITH ITS SENSOR TIP IS EXTRACTED FROM THE PROTECTIVE HOUSING, HANDLE WITH CARE. THE GLASS FIBER IS UNPROTECTED AND MIGHT BREAK!

7. Be carefully, by implanting the microsensor into your specially designed system. Please contact our service team for custom desingned systems.
8. Please rinse the glass fiber with its sensor tip with distilled water after removing it from the sample to remove any sample residues. Retract the sensor tip into the protective housing and insert the transport block to prevent the syringe plunger from slipping.

8.4 Some Advice for Correct Measurement

8.4.1 Signal drifts due to oxygen gradients

Please take into account that the sensor has a high spatial resolution. An oxygen gradient occurs most times in unstirred solutions which are in contact with ambient air.

In case of needle type sensors, check first if the tip is completely extended from the needle or if air bubbles are on the sensor tip whenever unexpected drifts, gradients or unstable measurement values occur.

In case of flow trough cells, air bubbles located at the sensor tip cause signal drifts.

Critical conditions for bubble formations are, for example, purging with air or other gases and increasing temperature during measurement.

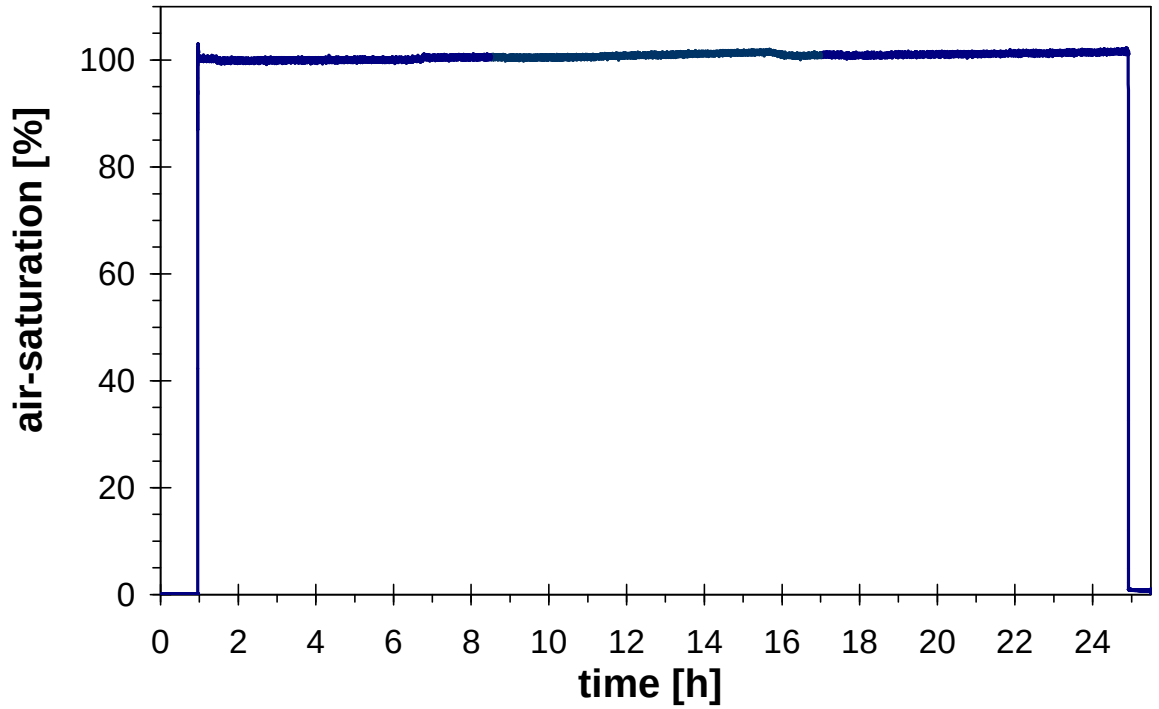
The formation of a bio-film during long term measurements or the accumulation of other sample components like oil or solid substances may also lead to an oxygen gradient.

8.4.2 Signal drifts due to temperature gradients

A further source of imprecise measurement is insufficient temperature compensation. If you use the temperature compensation, ensure that no temperature gradients exist between the microsensor and the temperature sensors. If you measure without temperature compensation, please bear in mind, that the Microx TX3 only measures correctly, if the sample temperature is constant during measurement and the temperature is the same as you typed in at the beginning of the measurement. Please also refer to Chapter 13.5 "Formulas for temperature compensation". If the temperature is measured with a precision of ± 0.2 °C, there is a variation in the measuring value at 100% air-saturation of 100 ± 0.3 % air-saturation. Please choose the measurement with temperature compensation to minimize temperature gradients.

8.4.3 Signal drift due to photo-decomposition

The oxygen-sensitive material may be subject to photo-decomposition resulting in a signal drift. Photo-decomposition takes place only during illumination of the sensor tip and depends on the intensity of the excitation light. Therefore, the excitation light is minimized.
Continuous illumination of a tapered sensor tip over a period of 24 hours may lead to a phase drift of up to + 1.6 % air-saturation measured at 100% air-saturation at 20°C. However, this effect of photo-decomposition can even be minimized, by changing the measuring mode to the second or minute interval mode. In these modes, the software switches off the excitation light after recording the data point and switches it on after the interval you have chosen.
Please use the interval method whenever it is possible to increase the shelf life of the microsensor.



Photostability of a tapered oxygen microsensor.

Drift in % air-saturation at 100% air-saturation when illuminating the microsensor with a tapered and flat broken sensor tip for 1, 12 and 24 hours in the continuous mode

Mode	Drift per hour	Drift per 12 hours	Drift per 24 hours
tapered sensor tip			
continuous mode (1 s)	± 0.6 % air-saturation	+1 % air-saturation	+1.6 % air-saturation
flat broken sensor tip			
	± 0.5% air-saturation	± 0.5% air-saturation	< 0.6 % air-saturation

8.4.4 Performance proof

If you want to prove the performance during the past measurement, please check the calibration values by inserting the sensor tip in the '**cal 0**' and '**cal 100**' calibration standards when you have finished your measurement. If the device shows 0% air-saturation immersing the sensor tip into the '**cal 0**' solution and 100 % air-saturation measuring the '**cal 100**' standard, the sensor worked perfectly during the whole measurement.

9 General Instructions:

9.1 Warm-Up Time

The warm up time of the electronic and opto-electronic components of the Microx TX3 is 5 min. Afterwards stable measuring values are obtained.

9.2 Maintenance

The instrument is maintenance-free.
The housing should be cleaned only with a moist cloth. Avoid any moisture entering the housing! Never use benzine, acetone, alcohol or other organic solvents.
The ST-fiber connector of the microsensor can be cleaned only with lint-free cloth. The sensor tip may be rinsed only with distilled water. Please ensure, that no sample residues are inside the syringe needle. If necessary, rinse the glass-fiber with its oxygen-sensitive tip with distilled water.

9.3 Service

Balancing, maintenance and repair work may only be carried out by the manufacturer:

Loligo Systems ApS
Niels Pedersens Allé 2
DK-8830 Tjele
Denmark

Phone: +45 8999 2545
E-mail: mail@loligosystems.com
Internet: www.loligosystems.com

Please contact our service team should you have any questions. We look forward for helping you and are open for any questions and criticism.

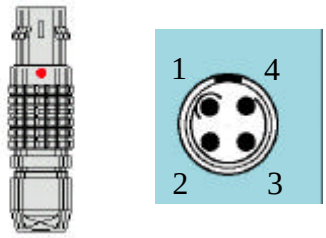
10 Technical Data

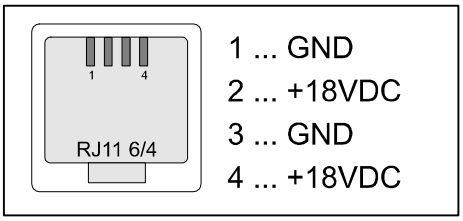
10.1 General Data

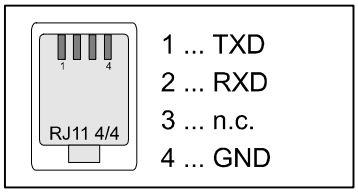
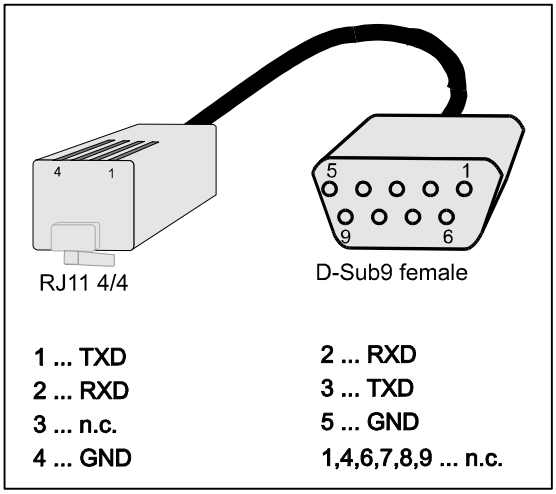
MODES	
oxygen	range: 0 - 500 % air-saturation resolution: 30 ± 0.3 % air -aturation 100 ± 0.9 % air-saturation 250 ± 3.6 % air-saturation accuracy: ± 1 % air-saturation
temperature	range: 0 - 50 °C resolution: ± 0.5 °C accuracy: ± 1° C

CALIBRATION PROCEDURE	2-point calibration in oxygen free water and humidified air or air-saturated water

OPTICAL OUTPUT / INPUT	
Optical connector	ST compatible, Core/Center 100/140
Channels	1
Wavelength	505 nm

TEMPERATURE SENSOR INPUT	
Lemo Connector Size 00	Connector for Pt-1000 temperature sensor  **1 ... PT1000-1** **2 ... n.c.** **3 ... n.c.** **4 ... PT1000-2**

DC INPUT	DC-Range : 12 V/1250mA up to 18V/900mA 

DIGITAL OUTPUT	
communication protocoll	serial interface RS232 19200 Baud, Databits 8, Stoppbits 1, Parity none, Handshake none
instrument output:	on RJ11 4/4 plug 
Interface cable to PC:	RJ11 4/4 to DSub9: 

ENVIRONMENTAL CONDITIONS	
Operating temperature	0 to +50ºC
Storage temperature	-10 to +65ºC
Relative humidity:	up to 95%

OPERATION CONTROL	*LED at the front panel:* red: instrument off green: instrument on orange: stand by

DIMENSIONS	length: 185 mm; width: 110 mm; height: 45 mm; weight: 630 g;

10.2 Analog Output and External Trigger

The TX3-AOT –instrument version is supplied with a dual programmable 12bit analog output with galvanic isolation and an external trigger input.

- **ANALOG OUTPUT**

GENERAL SPECIFICATION - ANALOG OUTPUT

Channels	2
Connector	BNC
Resolution	12 bit
Output range	0 to 4095mV (±2mV max. error)
Galvanic isolation	500V rms
Shortcut protection	Yes
Programmable to	oxygen, temperature, amplitude, phase by software

Equivalence coefficients :

oxygen	1 :: 0.1	(i.e. : 973 mV = 97.3 % air saturation)
temperature	1 :: 0.1	(i.e. : 208 mV = 20.8°C)
amplitude	1 :: 10	(i.e. : 2220 mV = 22200 relative units)
phase	1 :: 0.025	(i.e. : 1100 mV = 27.50°)

Update rate:
The update rate is dependent on the sampling rate of the software.
If an external trigger is used, the update rate is equivalent to the trigger pulse rate.

DC SPECIFICATION - ANALOG OUTPUT

Resolution

oxygen	± 2mV ➔ ± 0.2 % air saturation
temperature	± 2mV ➔ ± 0.2°C
amplitude	± 2mV ➔ ± 20 relative units
phase	± 2mV ➔ ± 0.05°)

Accuracy error	± 10mV

- **EXTERNAL TRIGGER INPUT**

GENERAL SPECIFICATION - EXTERNAL TRIGGER INPUT

Channels	1
Connector	BNC
Input voltage range	TTL-compatible / up to 24V
Trigger mode	Low-High-Low (Input must be kept Low for at least 50µs)
Normal state	no current
Isolation	500V rms

Timing Specifications:

Min rise &fall time for trigger	15ns (see TTL-specification)
Max rise &fall time for trigger	2 ms
Min pulse length	3 ms
Min pause length	10 ms
Min periode length	13 ms

10.3 Technical Notes

Power Adapter

Microx TX3 should always be used with the original power adapter (110-220VAC/12VDC). As an alternative power source a battery can be used that meets the DC input voltage given in technical specification. The battery adapter cable is available as an additional accessory.

Analog Outputs

WARNING: The analog outputs are not protected against any input voltage! Any voltage applied to the analog outputs can cause irreversible damage of the circuit.

RS232 Interface

The unit uses special interface cable. Another cable can cause the unit's malfunction.

Optical Output (ST)

The ST connector is a high precision optical component. Please keep it clean and dry. Always use the rubber cap to close the output when not in use.

10.4 Operation Notes

Oxygen Measurement

To achieve the highest accuracy Microx TX3 should be warmed-up for 5min before starting the measurement. Please see the details of measurement process described in Microx TX3 manual.

Temperature Compensation

No other than supplied temperature sensor could be used with the unit. The use of any other temperature sensor can damage the oxygen meter.

11 Trouble Shooting

Error	Cause	Action
Device does not work and LED on the front panel is not lit	Device is not switched on No power supply	Switch on device with ON/OFF switch on the rear panel Connect power supply with device
Device does not work and LED on the front panel is on	No connection to PC	Check connection of the device to your PC (RS 232)
Temperature compensation failed, no temperature measurement possible	PT 1000 sensor is not connected properly PT 1000 Sensor is faulty	Check connection, Contact our service
Warning light: Amplitude: red	Microsensor is not connected properly ST-Connector is contaminated Sensor tip is damaged	Check connection of the ST-connector Clean connector with a soft, lint-free cloth rag Replace oxygen sensor; (Send the sensor back to us for re-coating service;
Warning light: Phase: red	phase angle out of limits	- check connection of the microsensor - replace microsensor
Warning light: overload: red	too much false light	- reduce false light - use optical-isolated microsensors
no cal	calibration failed Sensor was not in the right calibration standard sulfite solution has aged	- calibrate again - check calibration solutions Immerse Sensor in the proper calibration vessel Prepare fresh sulfite -solution
Strong signal fluctuations	Air bubbles at sensor tip The glass-fiber with its sensor tip is not extended from the syringe needle	Remove air bubbles by carefully tapping Completely extend the sensor

12 Concluding Remarks

Dear customer,
With this manual, we hope to provide you with an introduction to work with the Microx TX3 fiber-optic oxygen-meter.

This manual does not claim to be complete. We are endeavored to improve and supplement this Version.
We are looking forward to your critical review and to any suggestions you may have.

You can find the newest version at www.loligosystems.com

With best regards,

Your Loligo Team

13 Appendix

13.1 Basics in Optical Sensing of Oxygen

13.1.1 Dynamic Quenching of Luminescence

The principle of measurement is based on the effect of dynamic luminescence quenching by molecular oxygen. The following scheme explains the principle of dynamic luminescence quenching by oxygen.

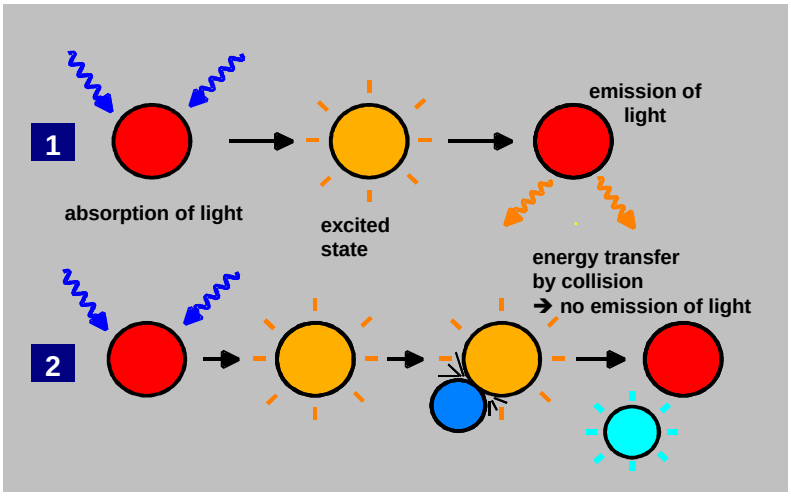


Figure 13.1 *Principle of dynamic quenching of luminescence by molecular oxygen*
(1) Luminescence process in absence of oxygen
(2) Deactivation of the luminescent indicator molecule by molecular oxygen

The collision between the luminophore in its excited state and the quencher (oxygen) results in radiationless deactivation and is called collisional or dynamic quenching. After collision, energy transfer takes place from the excited indicator molecule to oxygen which consequently is transferred from its ground state (triplet state) to its excited singlet state. As a result, the indicator molecule does not emit luminescence and the measurable luminescence signal decreases.
A relation exists between the oxygen concentration in the sample and the luminescence intensity as well as the luminescence lifetime which is described in the Stern-Volmer-equation (**1**). Here, τ_0 and τ are the luminescence decay times in absence and presence of oxygen (I_0 and I are the respective luminescence intensities), $[O_2]$ the oxygen concentration and K_{SV} the overall quenching constant

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2] \qquad (1)$$
$$I = f([O_2])$$
$$\tau = f([O_2])$$

I: Luminescence intensity in presence of oxygen
I_0: Luminescence intensity in absence of oxygen
τ: Luminescence decay time in presence of oxygen
τ_0: Luminescence decay time in absence of oxygen
K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)
$[O_2]$: oxygen content

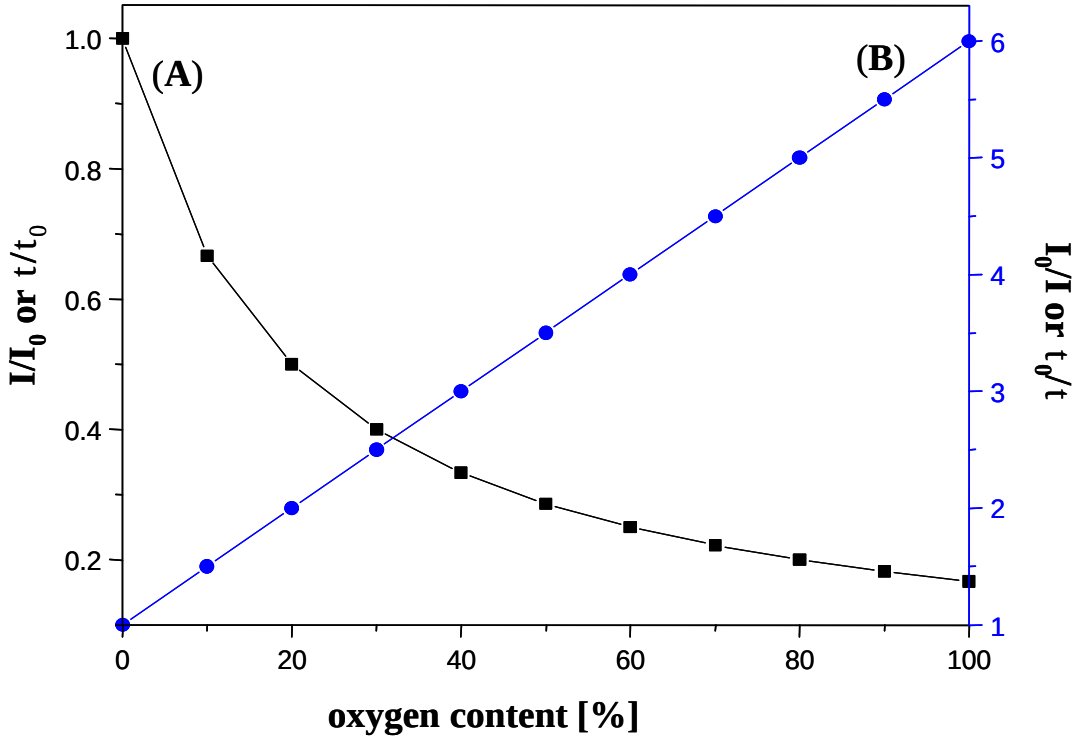


Figure. 13.2 *(**A**) Luminescence decrease in the presence of oxygen. (**B**) Stern-Volmer plot.*

Indicator dyes quenched by oxygen are, for example polycyclic aromatic hydrocarbons, transition metal complexes of Ru(II), Os(II) and Rh(II), and phosphorescent porphyrins containing Pt(II) or Pd(II) as the central atom.

13.1.2 Major Components of Fiber-Optic Microsensors

In optical chemical sensors, the analyte interacts with an indicator and changes its optical properties. The result is either a change in the color (absorbance or spectral distribution) or the luminescence properties (intensity, lifetime, polarisation). Light acts as the carrier of the information.

The major components of a typical fiber optical sensing system are

- a light source to illuminate the sensor (laser, light emitting diode, lamps)
- an optical fiber as signal transducer (plastic or glass fiber)
- a photodetector (photodiode, photomultiplier tube, CCD-array)
- the optical sensor (indicator immobilised in a solid matrix)

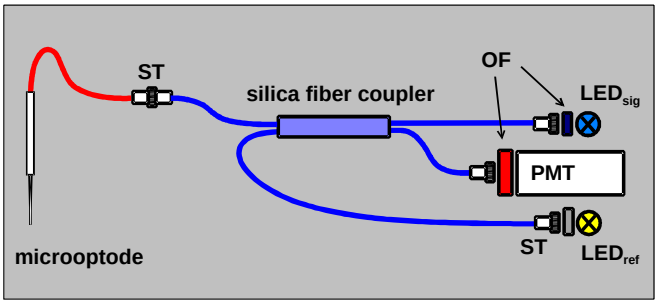


Figure 13.3 Schematic drawing of the optical setup of a measuring system with microsensors
(LED: light emitting diodes, PMT: photomultiplier, OF: optical filters, ST: fiber connector)

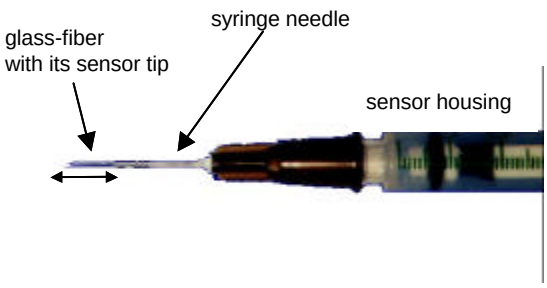


Figure 13.4 *Scheme of a microsensor*

13.1.3 Advantages of Optical Oxygen-Sensitive Microsensors

- no oxygen is consumed during the measurement;
- the signal is independent of changes in flow velocity;
- high spatial (< 50 µm) and temporal resolution (t_{90} < 1 s)
- they are able to measure the oxygen content in dry gases
- they are insensible towards electrical interferences and magnetic fields;
- they are more sensitive than conventional electrodes (up to ppb-range);
- using silica fibers, it is possible to measure in samples while physically separate from the light source and detectors;
- light conducting fibers are able to transport more information than power currents (information can be simultaneously transferred, e.g., intensity of light, spectral distribution, polarisation, information such as decay time or delayed fluorescence);

13.1.4 Luminescence Decay Time

The MICROX TX3 measures the luminescence decay time of the immobilised luminophore as the oxygen dependent parameter.

$$\tau = f([O_2]) \qquad (2)$$

The MICROX TX3 uses the phase-modulation technique to evaluate the luminescence decay time of the indicators. If the luminophore is exited with a sinusoidally intensity modulated light, its decay time causes a time delay in the emitted light signal. In technical terms, this delay is the phase angle between the exiting and emitted signal. This phase angle is shifted as a function of the oxygen concentration. The relation between decay time τ and the phase angle Φ is shown by the following equation:

$$\tau = \frac{\tan\Phi}{2\pi \cdot f_{mod}} \qquad (3a)$$

$$\tan\Phi = 2\pi \cdot f_{mod} \cdot \tau \qquad (3b)$$

$$\tau \equiv \tan\Phi \equiv \Phi \equiv f([O_2]) \qquad (3c)$$

τ: luminescence decay time; Φ: phase angle; f_{mod}: modulation frequency

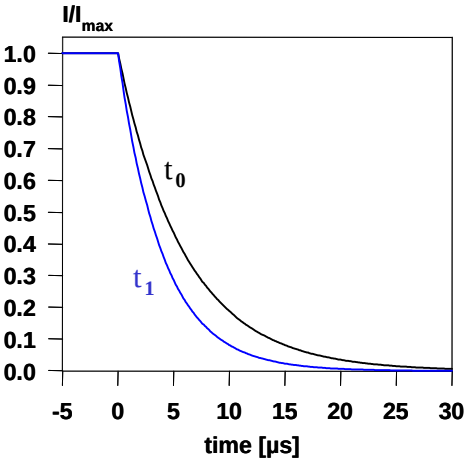


Figure 13.5 *Schematic of the single exponential decay ($t_0 > t_1$).*

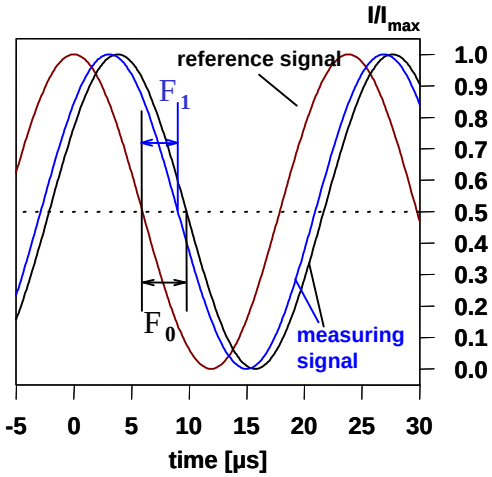


Figure 13.6 *The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state*

The measurement of the luminescence decay time, an intrinsically referenced parameter, has the following advantages compared to the conventional intensity measurement.

- The decay time does not depend on fluctuations in the intensity of the light source and the sensitivity of the detector;
- The decay time is not influenced by signal loss caused by fiber bending or by intensity changes caused by changes in the geometry of the sensor;
- The decay time is, to a great extent, independent of the concentration of the indicator in the sensitive layer ➔ Photobleaching and leaching of the indicator dye has less influence on the measuring signal;

The decay time is not influenced by variations in the optical properties of the sample including turbidity, refractive index and coloration.

13.1.5 Literature

If you want to find out more about this subject, we recommend the following publications.

- Wolfbeis O.S. (Ed.), ***Fiber Optic Chemical Sensors and Biosensors***, Vol. **1**&**2**, CRC, Boca Raton (1991).
- Klimant I., Wolfbeis O.S., ***Oxygen-Sensitive Luminescent Materials Based on Silicone-Soluble Ruthenium Diimine Complexes***, Anal. Chem., **67**, 3160-3166 (1995).
- Klimant I., Kühl M., Glud R.N., Holst G., ***Optical measurement of oxygen and temperature in microscale: strategies and biological applications***, Sensors and Actuators B, **38-39**, 29-37 (1997).
- Holst G., Glud R.N., Kühl M., Klimant I., ***A microoptode array for fine-scale measurement of oxygen distribution***, Sensors and Actuators B, **38-39**, 122-129 (1997).
- Klimant I., Meyer V., Kühl M., ***Fiber-optic oxygen microsensors, a new tool in aquatic biology***, Limnol. Oceanogr., **40**, 1159-1165 (1995).
- Klimant I., Ruckruh F., Liebsch G., Stangelmayer A., Wolfbeis O.S., ***Fast Response Oxygen Microsensors Based on Novel Soluble Ormosil Glasses***, Mikrochim. Acta, **131**, 35-46 (1999).

13.2 Determination of the Oxygen Concentration Using a Modified Stern-Volmer Equation

The Stern-Volmer equation (**4**) displays a linear correlation between $\tan\Phi_0/\tan\Phi$ or τ_0/τ and the oxygen concentration $[O_2]$.

$$\frac{\tan\Phi_0}{\tan\Phi} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2] \qquad (4)$$

Φ_0 phase angle of oxygen-free water
Φ measured phase angle
K_{SV} Stern-Volmer Constant
$[O_2]$ oxygen content in %-air-saturation

The Stern-Volmer plots of all our sensors show a distinct non-linearity in their response behavior which is also observed for many other oxygen sensors described in literature.
This non-linear response behavior can be described with a modified Stern-Volmer equation.

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{f_1}{1+K_{SV_1}\cdot[O_2]} + \frac{1-f_1}{1+K_{SV_2}\cdot[O_2]}) \right)^{-1} \quad (\mathbf{5})$$

This model is based on the assumption that the indicator is distributed in the polymer matrix at two different sites and each fraction (f_1, 1-f_1) shows a different quenching constant (K_{SV1}, K_{SV2}).

For practical use this model is not very convenient since it has too many parameters which have to be calibrated. Therefore, two simplified models based on equation 5 can be used.

In the first model, one fraction of the indicator is assumed to be non-quenchable (K_{SV2}=0).

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{f_1}{1+K_{SV}\cdot[O_2]} + (1-f_1) \right)^{-1} \quad (\mathbf{6})$$

Equation 7 was used to describe the oxygen calibration plot of a microsensor type B2. The correlation coefficient R^2 of this fit was higher than 0.999.

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{0.89}{1+K_{SV}\cdot[O_2]} + 0.11) \right)^{-1} \quad (\mathbf{7})$$

The oxygen content in %-air-saturation can be calculated according to equation 8.

$$[O_2] = \frac{1-\frac{\tan\Phi}{\tan\Phi_0}}{K_{SV}\cdot\left(\frac{\tan\Phi}{\tan\Phi_0}-0.11\right)} \quad (\mathbf{8})$$

A second model, which also is based on equation 5, can be used for describing the oxygen calibration plot. In this model, K_{SV2} is set to be x*K_{SV1}. f_1 was determined to be 0.805 and x was determined to be 1/22.9. The correlation coefficient R^2 of this fit was higher than 0.9999.

$$\frac{\tan\Phi_0}{\tan\Phi} = \left(\frac{0.804}{1+K_{SV_1}\cdot[O_2]} + \frac{0.196}{1+\frac{1}{22.9}\cdot K_{SV_1}\cdot[O_2]}) \right)^{-1} \quad (\mathbf{9})$$

The oxygen content in %-air-saturation can be calculated according to equation 10.

$$[O_2] = \frac{-B + \sqrt{B^2 - 4 \cdot A \cdot C}}{2 \cdot A} \quad (\mathbf{10})$$

with the coefficients

$$A = \frac{\tan \Phi}{\tan \Phi_0} \cdot x \cdot K_{SV}^2 \quad (\mathbf{10a})$$

$$B = \frac{\tan \Phi}{\tan \Phi_0} \cdot K_{SV} + \frac{\tan \Phi}{\tan \Phi_0} \cdot x \cdot K_{SV} - f_1 \cdot x \cdot K_{SV} - K_{SV} + f_1 K_{SV} \quad (\mathbf{10b})$$

$$C = \frac{\tan \Phi}{\tan \Phi_0} - 1 \quad (\mathbf{10c})$$

13.3 Oxygen Conversion Formulas

Please note:
These conversion formulas are only valid in aqueous solutions and humidified air. These formulas have to be modified if measurements are performed in organic solvents or solutions with high salinity.

% -Saturation

%-air saturation

Default-Setting of the instrument (see equation 10 in 13.2)

%-oxygen saturation

$$\%O_2 = \%\ \text{air - saturation} \cdot \frac{20.95}{100} \quad (\mathbf{11})$$

0.2095: volume content of oxygen in air

ppm in the gaseous phase:

$$\text{ppm}[O_2] = \%\text{air} - \text{saturation} \cdot \frac{20.95}{100} \cdot 10000 \quad (\mathbf{12})$$

$$1\text{ppm} = \frac{1}{1000000} = \frac{1\text{mg}}{1\text{kg}} = \frac{1\mu\text{L}}{1\text{L}} = \frac{1}{10000}\%$$

Partial pressure of oxygen

in hPa

$$p_{O_2}[\mathrm{hPa}] = \left(p_{atm}[\mathrm{hPa}] - p_W(T)[\mathrm{hPa}]\right) \cdot \frac{\%\ \text{air - saturation}}{100} \cdot 0.2095 \quad (\mathbf{13})$$

in mbar

$$p_{O_2}[mbar] = \left(p_{atm}[mbar] - p_W(T)[mbar]\right) \cdot \frac{\%\ \text{air - saturation}}{100} \cdot 0.2095 \quad (\mathbf{14})$$

in Torr

$$p_{O_2}[Torr] = \left[\left(p_{atm}[mbar] - p_W(T)[mbar]\right) \cdot \frac{\%\ \text{air - saturation}}{100} \cdot 0.2095\right] \cdot 0.75 \quad (\mathbf{15})$$

Please note:
1 mbar = 1 hPa = 0.750 Torr

Oxygen Concentration

in mg/L

$$c_{O_2}[\mathrm{mg/L}] = \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air - saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \quad (\mathbf{16})$$

in ppm

$$c_{O_2}[\mathrm{ppm}] = \frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air - saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M} \quad (\mathbf{17})$$

in µmol/L

$$c_{O_2}[\mu\mathrm{mol/L}] = \left[\frac{p_{atm} - p_W(T)}{p_N} \cdot \frac{\%\ \text{air - saturation}}{100} \cdot 0.2095 \cdot \alpha(T) \cdot 1000 \cdot \frac{M(O_2)}{V_M}\right] \cdot 31.25 \quad (\mathbf{18})$$

p_{atm}: actual atmospheric pressure;

p_N: standard pressure (1013 mbar);

0.2095: volume content of oxygen in air;

$p_W(T)$: vapor pressure of water at temperature T given in Kelvin;

$\alpha(T)$: Bunsen absorption coefficient at temperature T;

$M(O_2)$: molecular mass of oxygen (32 g/mol);

V_M: molar volume (22.414 l/mol);

13.4 Temperature Dependent Constants Affecting the Oxygen Content

13.4.1 Water Vapor Pressure

As shown in equation 13 - 18, the water vapor pressure p_w influences the oxygen partial pressure of air-saturated water and water-vapor saturated air.

Oxygen partial pressure in dry air:

$$p(O_2) = p_{atm} \cdot 0.2095 \quad (19)$$

$p(O_2)$: oxygen partial pressure in dry air at a barometric pressure p_{atm}
0.2095: volume content of oxygen in air;

Oxygen partial pressure in air-saturated water and water-vapor saturated air:

$$p(O_2)' = \left(p_{atm} - p_W(T)\right) \cdot 0.2095 \quad (20)$$

Water vapor pressure is strongly affected by temperature variations and this, of course, influence the oxygen partial pressure as shown in equation 20.

Table 13.1 Variation of water vapor pressure $p_W(T)$ with temperature.

q **[°C]**	0	5	10	15	20	25	30	35	40	50
T **[K]**	273	278	283	288	293	298	303	308	131	323
p_W(T) [mbar]	6.1	8.7	12.3	17.1	23.3	31.7	42.4	56.3	73.7	123.3

A convenient fitting function is given by the Campbell equation 21

$$p_W(T) = \exp\left(A - \frac{B}{T} - C \cdot \ln T\right) \quad (21)$$

where T is the temperature in Kelvin and A, B and C constants given in Figure 13.7

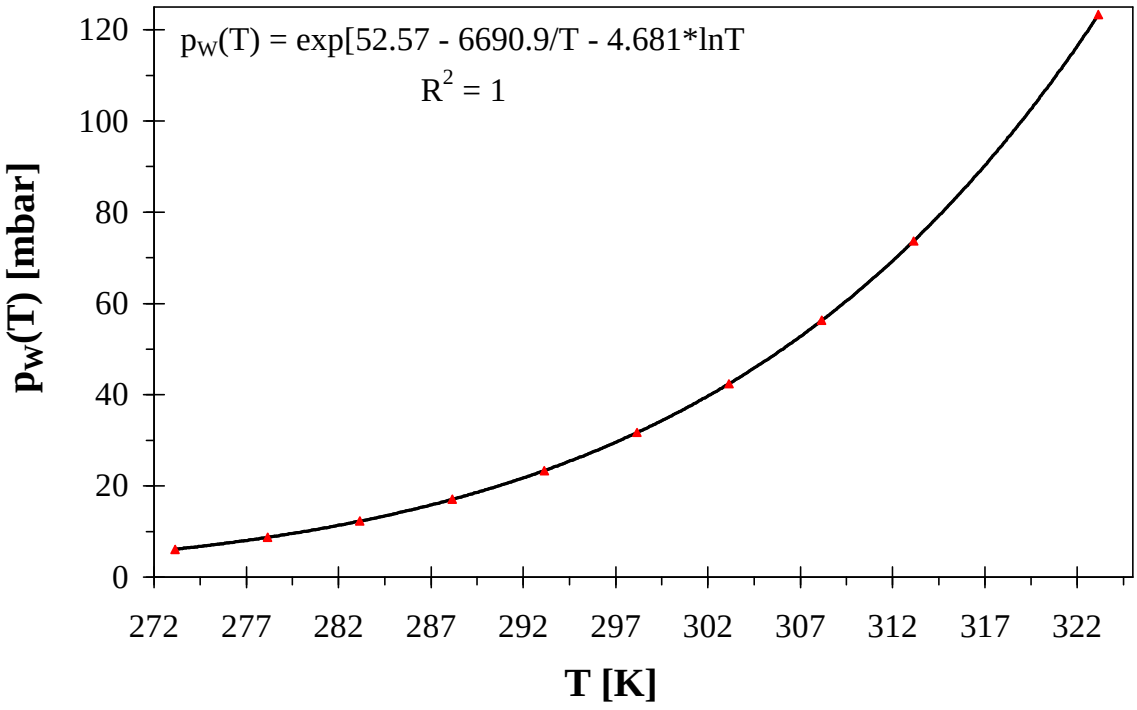


Figure. 13.7 *Variation of water vapor pressure with temperature.*

13.4.2 Bunsen Absorption Coefficient

The solubility of oxygen in water is temperature dependent and can be described using the Bunsen absorption coefficient $\alpha(\theta)$ and the oxygen partial pressure $p(O_2)$ according to equation 22. With increasing temperature, the solubility of oxygen in water decreases.

$$c_S(p,\theta) = \frac{p(O_2)}{p_N}\alpha(\theta) \qquad (22)$$

$c_S(p,\theta)$: temperature dependent solubility of oxygen in water
$p(O_2)$: oxygen partial pressure
p_N: standard pressure (1013 mbar);

Table 13.2 Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature.

q **[°C]**	0	5	10	15	20	25	30	35	40	50
a**(q)·10^3**	49.01	42.94	38.11	34.17	31.01	28.43	26.30	24.63	23.16	20.85

The data in Table 13.2 can be described by two forms of equations.

The first form of equation to describe the temperature dependent variation of the Bunsen absorption coefficient $\alpha(\theta)$ is obtained by fitting a general power series to the values in Table 13.2. A fourth degree polynomial fit can be chosen, yielding equation 23

$$10^3\alpha = a + b\cdot\theta + c\cdot\theta^2 + d\cdot\theta^3 + e\cdot\theta^4 \qquad (23)$$

where θ is the temperature in °C and a - e the coefficients calculated by standard curve fitting procedures.

a = 48.998
b = -1.335
c = 2.755 * 10^{-2}
d = -3.220 * 10^{-4}
e = 1.598 * 10^{-6}

The square of the correlation coefficient is 0.999996.

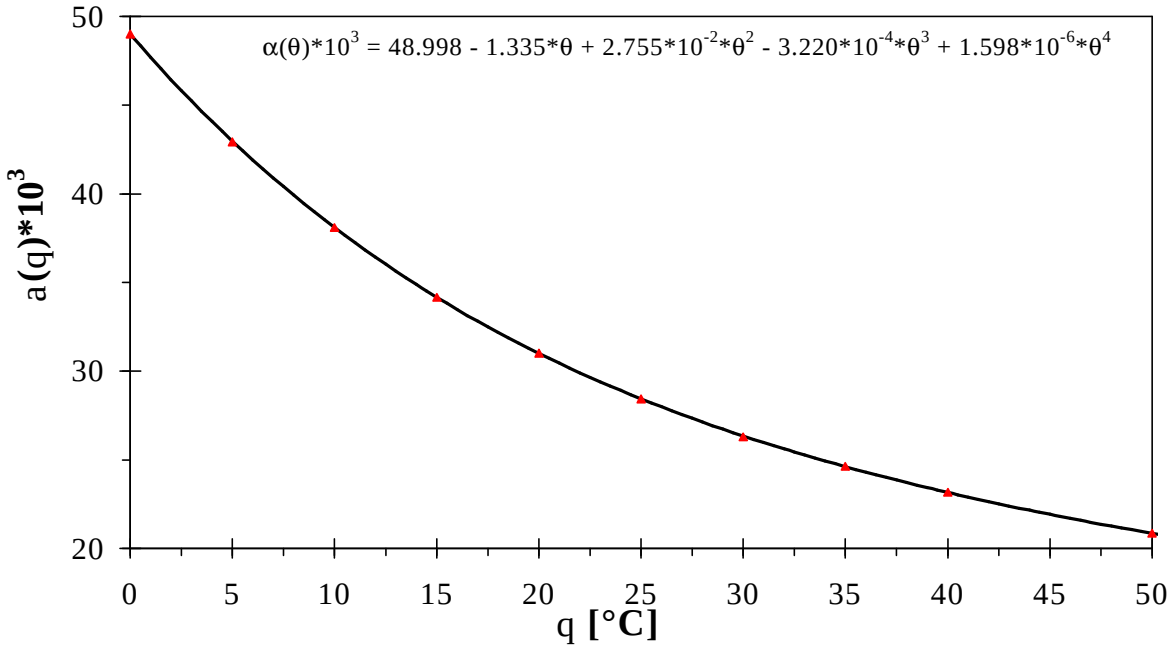


Figure 13.8 Variation of Bunsen absorption coefficient $\alpha(\theta)$ with temperature.

The other form of equation to describe the variation of α with temperature can be derived from a thermodynamical correlation and gives an equation of the form

$$\ln 10^3 \alpha = \frac{A}{T} + B \cdot \ln T + C \qquad (\mathbf{24})$$

where A, B and C are constants and T is the temperature in K. For oxygen dissolved in water we find by fitting the equation to the values of α in Table 13.2 that A = 8.553 * 10^3, B = 2.378 * 10, and C = -1.608 * 10^2.

Values of α calculated from eqns. 23 and 24 for the same temperature agree within ±0.5%.

The Bunsen absorption coefficient, however, is not a very practical measure. Values of $\alpha(\theta)$ have therefore to be converted to mg/L, and the method for doing this is best illustrated by an example.

Example: Calculation of the oxygen content ($c_S(p_{atm},\theta)$ in air-saturated water at a temperature θ of 20°C.
Equation 24 allows the solubility of oxygen in air-saturated fresh water to be calculated for any temperature and pressure provided that the values of the Bunsen absorption coefficient $\alpha(T)$ and the vapor pressure $p_W(T)$ at the particular temperature are known. Equation 23 or 23 can be used to obtain α, and p_W can be calculated from equation 21. Table 13.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C.

$$c_S(p_{atm},\theta) = \frac{p_{atm} - p_W(\theta)}{p_N} \cdot 0.2095 \cdot \alpha(\theta) \cdot \frac{M_{O_2}}{V_M} \qquad (\mathbf{25})$$

In equation 25 p_{atm} is the actual atmospheric pressure corrected for the contribution of the water vapor pressure p_w and related to standard pressure p_N. The corrected pressure is multiplied by 0.2095, the volume content of oxygen in air, by $\alpha(\theta)$ and by the molecular mass of oxygen (M_{O2}) divided by the molar volume V_M.
At a given atmospheric pressure of 1013 mbar (p_{atm} = p_N) and a temperature of 20 °C the oxygen content can be calculated according to equation 26.

$$c_S(1013\text{mbar},20°\text{C}) = \frac{1013 - 23.3}{1013} \cdot 0.2095 \cdot 0.031 \cdot \frac{32 \cdot \text{g/mol}}{22.414\text{mol/L}} = 0.009\,\text{g/L} = 9.06\,\text{mg/L} \qquad (\mathbf{26})$$

Figure 13.9 shows the temperature-dependent oxygen solubility in air-saturated fresh water and Table 13.3 gives oxygen solubilities in mg/L for temperature intervals of 0.1 °C from 0-40°C, as calculated via equation 25 and 23 for the Bunsen absorption coefficient.

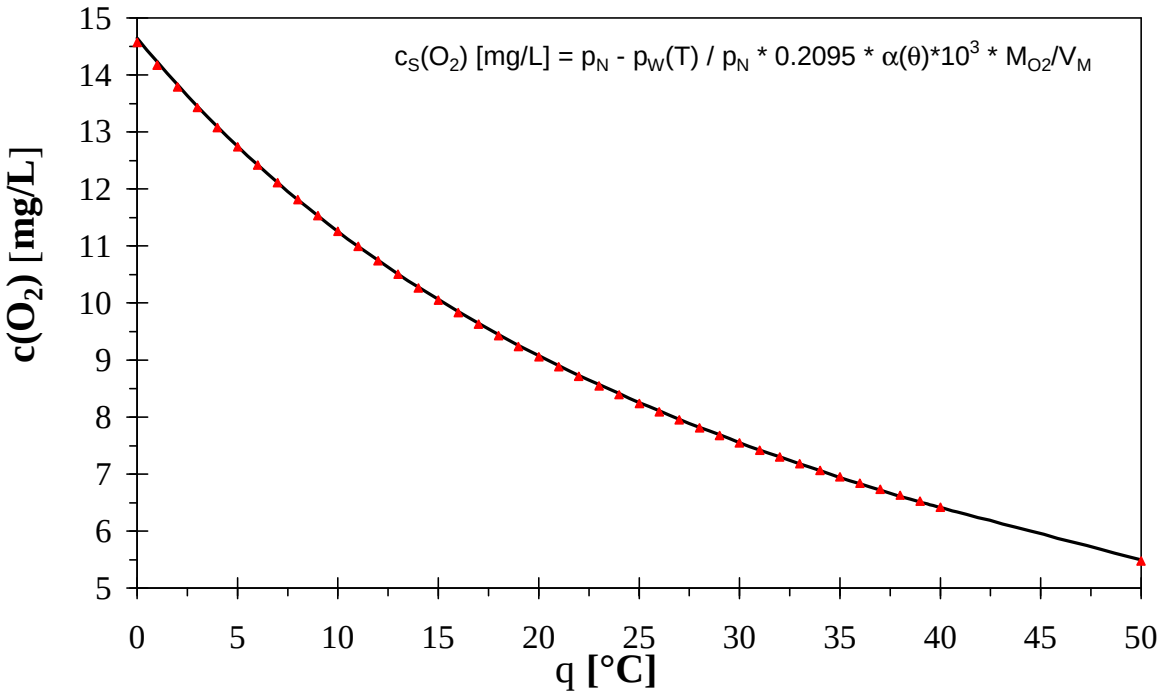


Figure 13.9 *Dependence of the oxygen solubility in air-saturated fresh water on temperature*

Table 13.3 Oxygen solubility in air-saturated fresh water [mg/L].

T [°C]	c_S(T)	.0	.1	.2	.3	.4	.5	.6	.7	.8	.9	1.0
0	**14.**	64	60	55	51	47	43	39	35	31	27	23
1		23	19	15	10	06	03	99	95	91	87	83
2	**13.**	83	79	75	71	68	64	60	56	52	49	45
3		45	41	38	34	30	27	23	20	16	12	09
4		09	05	02	98	95	92	88	85	81	78	75
5	**12.**	75	71	68	65	61	58	55	52	48	45	42
6		42	39	36	32	29	26	23	20	17	14	11
7		11	08	05	02	99	96	93	90	87	84	81
8	**11.**	81	78	75	72	69	67	64	61	58	55	53
9		53	50	47	44	42	39	36	33	31	28	25
10		25	23	20	18	15	12	10	07	05	02	99
11	**10.**	99	97	94	92	89	87	84	82	79	77	75
12		75	72	70	67	65	63	60	58	55	53	51
13		51	48	46	44	41	39	37	35	32	30	28
14		28	26	23	21	19	17	15	12	10	08	06
15		06	04	02	99	97	95	93	91	89	87	85
16	**9.**	85	83	81	70	76	74	72	70	68	66	64
17		64	62	60	58	56	54	53	51	49	47	45
18		45	43	41	39	37	35	33	31	30	28	26
19		26	24	22	20	19	17	15	13	11	09	08
20		08	06	04	02	01	99	97	95	94	92	90
21	**8.**	90	88	87	85	83	82	80	78	76	75	73
22		73	71	70	68	66	65	63	62	60	58	57
23		57	55	53	52	50	49	47	46	44	42	41
24		41	39	38	36	35	33	32	30	28	27	25
25		25	24	22	21	19	18	16	15	14	12	11
26		11	09	08	06	05	03	02	00	99	98	96
27	**7.**	96	95	93	92	90	89	88	86	85	83	82
28		82	81	79	78	77	75	74	73	71	70	69
29		69	67	66	65	63	62	61	59	58	57	55
30		55	54	53	51	50	49	48	46	45	44	42
31		42	41	40	39	37	36	35	34	32	31	30
32		30	29	28	26	25	24	23	21	20	19	18
33		18	17	15	14	13	12	11	09	08	07	06
34		06	05	04	02	01	00	99	98	97	96	94
35	**6.**	94	93	92	91	90	89	88	87	85	84	83
36		83	82	81	80	79	78	77	75	74	73	72
37		72	71	70	69	68	67	66	65	64	63	61
38		61	60	59	58	57	56	55	54	53	52	51
39		51	50	49	48	47	46	45	44	43	42	41
40		41	40	39	38	37	36	35	34	33	32	31

Example:: c_S(**14.3°C**) = **10.21 mg/L**

13.4.3 Dependence on the Salt Concentration

Table 13.4 gives values of the concentration of dissolved oxygen at several temperatures in solutions with various chloride concentrations. Increasing the salt concentration, there is a decrease in the oxygen solubility. This behavior is characteristic for the solubility of many nonelectrolytes - it is the phenomenon known as the ***salting-out effect***.

Instead of chlorinity [Cl^-] - the amount of chloride in parts per thousand - which was used as a measure of the amount of salt in water, the term salinity is often used. If salinity is preferred as a measure of salt concentration, then the conversion from g/L can be readily made using equation 27.

$$S = 1.805[Cl^-] + 0.03 \quad (27)$$

where S is the salinity in [‰] or [g/1000g]

Table 13.4 Solubility of oxygen in water as a function of temperature and salt concentration (Total pressure = 760 torr)

[Cl⁻] (g/1000g)	0	4	8	12	16	20
T [°C]	***Oxygen solubility [mg/L]***					
0	14.5	13.9	13.3	12.6	12.0	11.3
10	11.3	10.8	10.4	9.9	9.5	9.0
20	9.1	8.8	8.5	8.1	7.8	7.4
30	7.5	7.3	7.0	6.7	6.4	6.1

The effect of increasing the salt concentration on the vapor pressure is neglectible small as shown in Table 13.5.

Table 13.5. Variation of solution vapor pressure (p_W) with salt concentration

[Cl⁻] (g/1000g)	0	9	18	26
T [°C]	***Vapor pressure of solution (torr)***			
0	4.6	4.5	4.4	4.4
10	9.2	9.1	8.9	8.8
20	17.5	17.3	17.0	16.7
30	31.8	31.4	30.9	30.4

The dependence of oxygen solubility on salt concentration can also be obtained from equation 21 except that now values calculated from either equation 28 or 29 have to be used.

$$10^3 \cdot \alpha = a + b \cdot \theta + c \cdot \theta^2 + d \cdot \theta^3 + e \cdot \theta^4 - [Cl^-] \cdot (p + q \cdot \theta + r \cdot \theta^2 + s \cdot \theta^3 + t \cdot \theta^4) \quad (28)$$

where θ is the temperature in °C, a - e are the coefficients used in equation 23 and p - t are new constants given in Table 13.6. The values of these new constants, obtained by fitting the polynomial to experimental data in the ranges $0 \le \theta \le 30$°C and $0 \le [Cl^-] \le 20$‰. To obtain an oxygen solubility from the Bunsen absorption coefficient the same procedure as described previously is used.

An alternative equation to compensate the Bunsen absorption coefficient by the salt concentration displays equation 29.

$$10^3 \cdot \alpha = \exp\left[\left(A + \frac{B}{T} + C \cdot \ln T + D \cdot T\right) - [Cl^-]\left(P + \frac{Q}{T} + R \cdot \ln T + S \cdot T\right)\right] \quad (\mathbf{29})$$

where T is the temperature in Kelvin, and A - D and P - S are the coefficients also given in Table 13.6. They are based on measurements for 273.1 ≤ T ≤ 308.18 K and 0 ≤ [Cl⁻] ≤ 30‰ and is therefore more extensive than equation **28**. Both equations give values of $10^3\alpha$ which agree to better than ±1%.

Table 13.6 Values of the coefficients in equations **26** and **27**.

Eqn. 26	a	4.900 * 10	p	$5.516 * 10^{-1}$
	b	-1.335	q	$-1.759 * 10^{-2}$
	c	$2.759 * 10^{-2}$	r	$2.253 * 10^{-4}$
	d	$-3.235 * 10^{-4}$	s	$-2.654 * 10^{-7}$
	e	$1.614 * 10^{-2}$	t	$5.362 * 10^{-8}$
Eqn. 27	A	-7.424	P	$-1.288 * 10^{-1}$
	B	$4.417 * 10^3$	Q	5.344 * 10
	C	-2.927	R	$-4.442 * 10^{-2}$
	D	$4.238 * 10^{-2}$	S	$7.145 * 10^{-4}$

Seawater has a salinity typically of 35‰ (35g / 1000g) or a chloride content of about 19‰, and therefore, falls within the scope of both equations.

13.5 Temperature Compensation of the Response of Oxygen Sensors

Temperature affects the luminescence decay time as well as the luminescence intensity of the indicator dye. The collisional frequency of the oxygen molecules with the indicator dye reflecting the diffusion coefficient of oxygen is also affected.
A typical oxygen response characteristic at different temperatures is shown in Figure 13.10. The phase angle Φ is a function of the oxygen content (Φ = tanΦ = f(O_2)) and decreases with increasing the oxygen content.

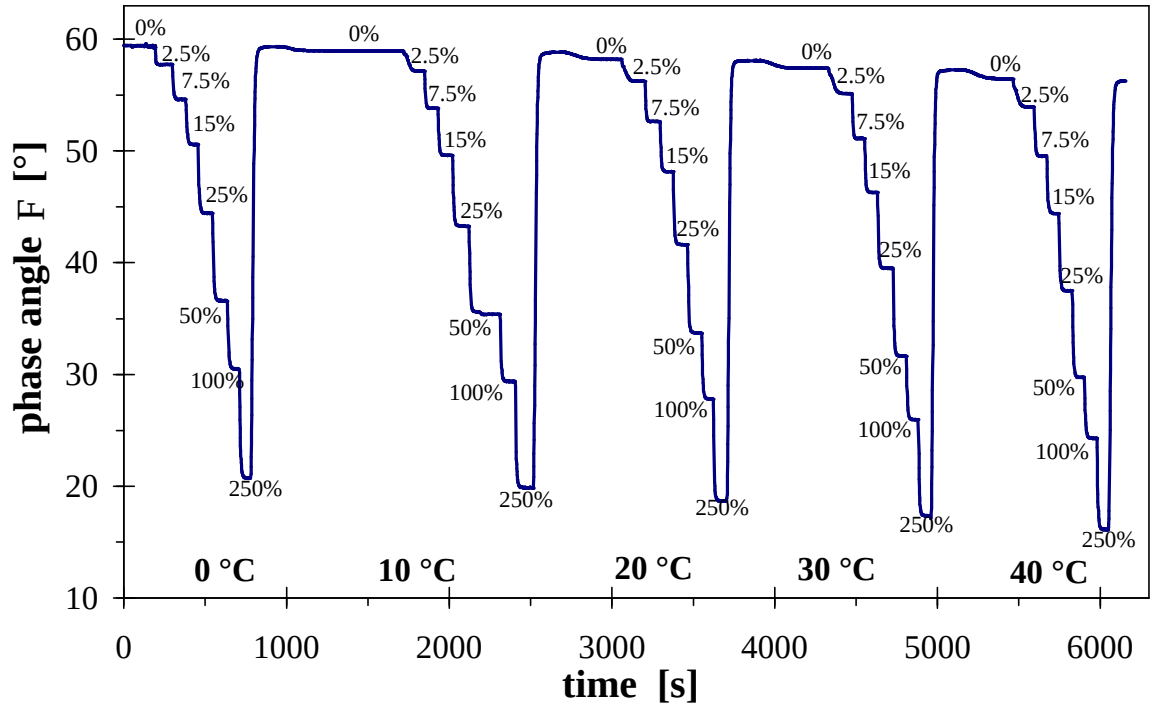


Figure 13.10 *Oxygen response characteristics at different temperatures.*

Figure 13.11 displays the oxygen-dependence of the phase angle at different temperatures and Figure 13.12 the respective Stern-Volmer plots. These two figures and Table 13.7 displays, that both the phase angle in absence of oxygen, Φ_0, and the Stern-Volmer constant, K_{SV}, are temperature dependent. Φ_0 decreases with increasing temperature, while KSV increases with increasing temperature.

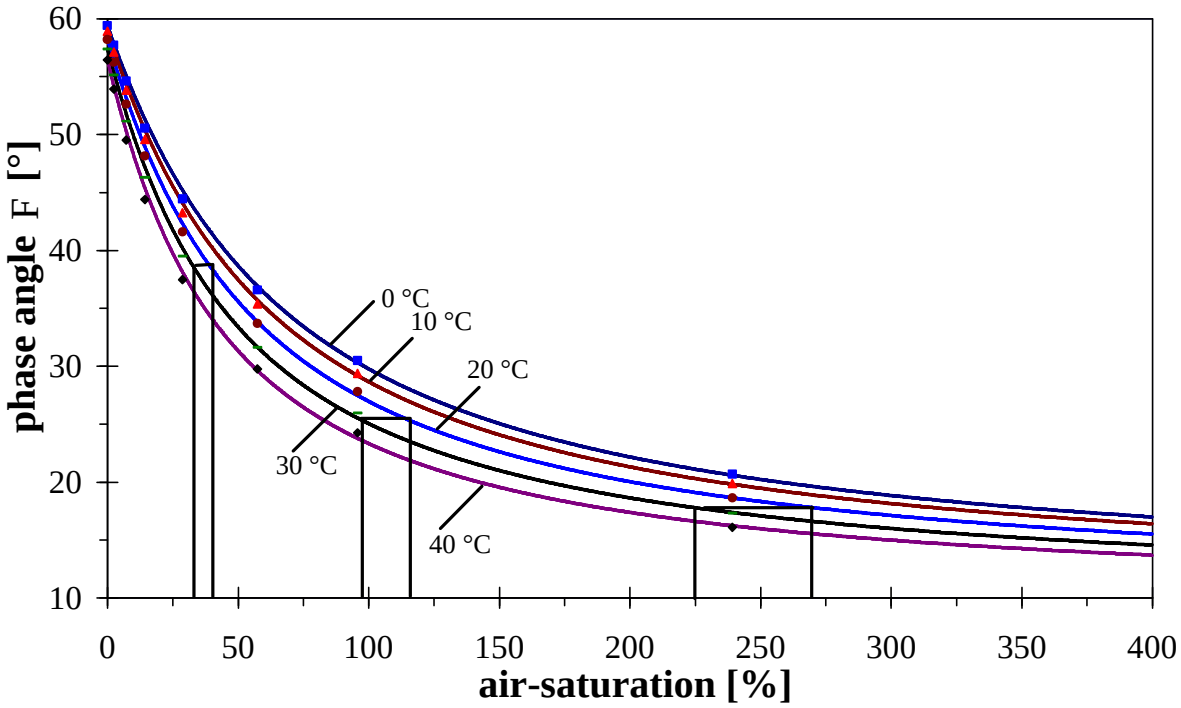


Figure 13.11 *Effect of the temperature on the phase angle at different oxygen contents given in % air-saturation.*

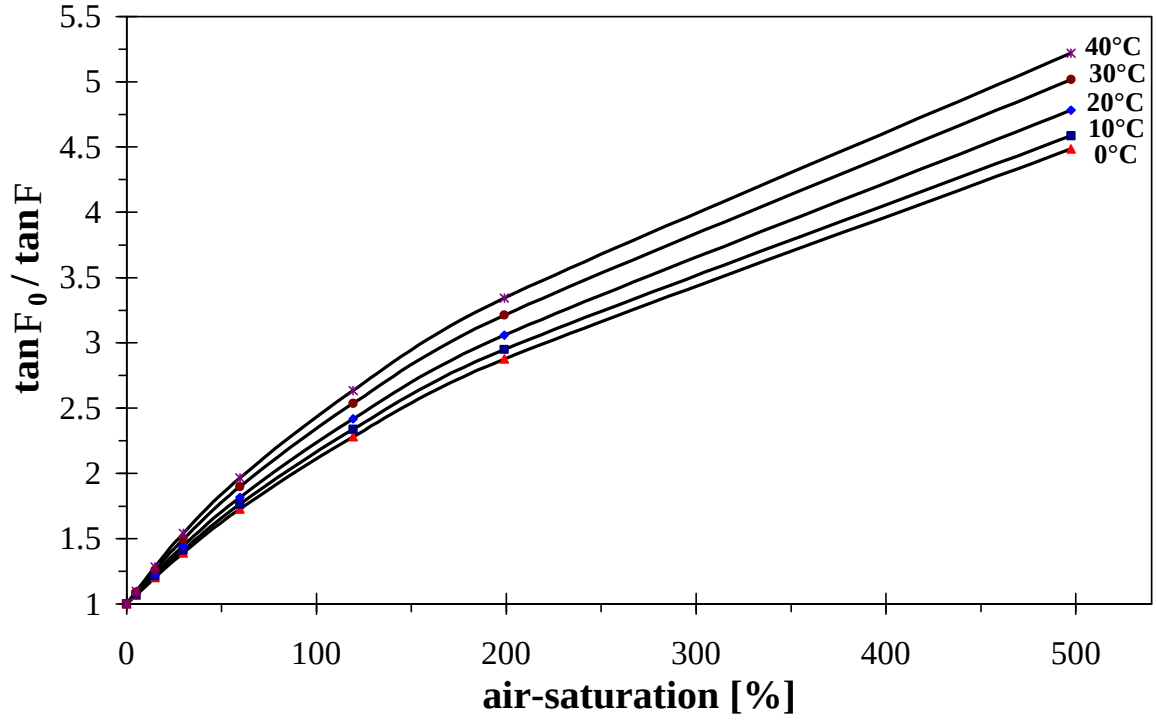


Figure 13.12 *Effect of the temperature on the Stern-Volmer constant.*

Table 13.7 Effect of the temperature on the phase angle in the absence of oxygen (Φ_0) and the Stern-Volmer constant K_{SV}.

q [°C]	*3.5*	*10*	*20*	*30*	*40*
F_0 [°]	59.43	58.94	58.2	57.4	56.44
K_{SV}[a] [% air-sat]$^{-1}$	0.03678	0.03929	0.04257	0.04698	0.05085

[a]: The Stern-Volmer constant is determined via equation 9.

Figure 13.13 displays the temperature dependence of Φ_0 and K_{SV}. From Table 13.7 and Figure 13.13 a decreases in Φ_0 of about 0.08° can be calculated by increasing the temperature by 1 K. On the other hand, the Stern-Volmer constant (K_{SV}) increases about $3.8*10^{-4}$ [% air-sat.]$^{-1}$ by increasing the temperature by 1 K.

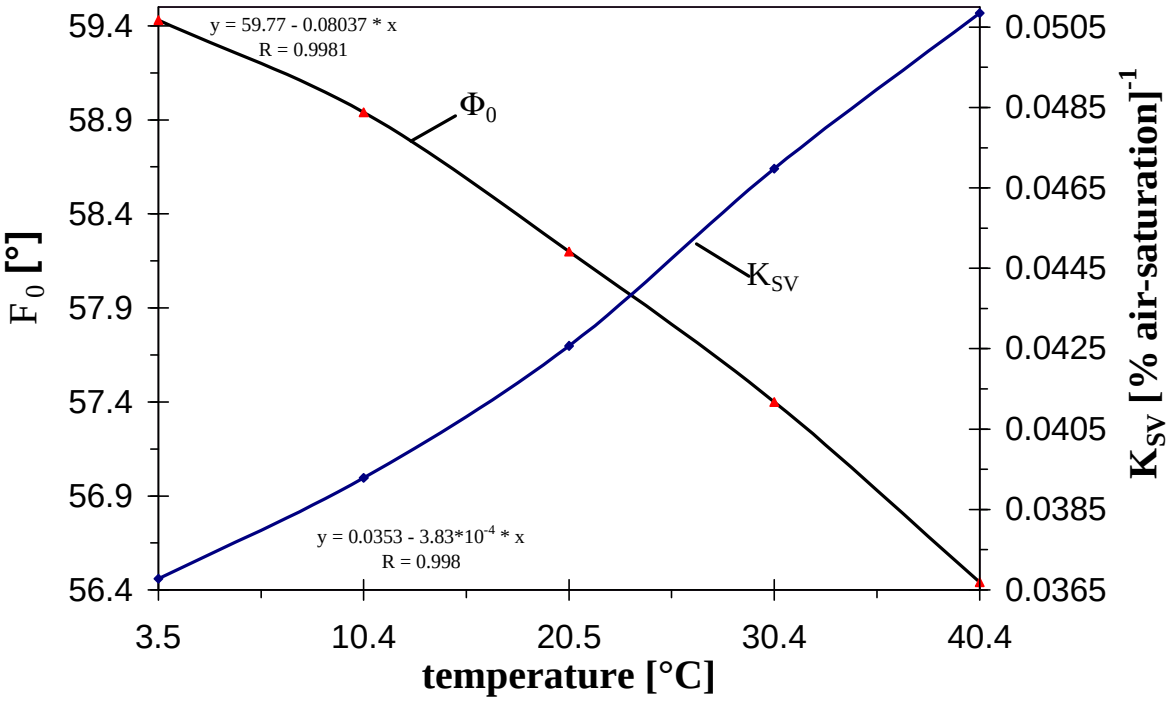


Figure 13.13 *Effect of the temperature on F_0 and the Stern-Volmer consant (K_{SV}).*

Consequently, variations in the temperature causes variations in the measuring value at a constant oxygen content (see Figure 13.11). Table 13.8 displays the deviation of the measuring value from the real oxygen content depending on temperature and the oxygen content.

Tabelle 13.8 Variation of the measured oxygen content (Δ % air-saturation) at a constant oxygen content and variations in temperature by ±1K.

% air-saturation	30 %	100 %	250 %
Δ%air-saturation /K	± 0.56 %	± 1.72 %	± 4.68 %

Example:

If the temperature is measured with a precision of ± 0.2 °C, there is a variation in the measuring value at 100% air-saturation of 100 ± 0.3 % air-saturation.